TWENTY-SEVENTH ANNUAL MEETING OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

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Headquarters, Hotel Loraine

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GENERAL BACTERIOLOGY

1. Some Fallacious Tendencies in Bacteriologic Taxonomy. IVAN C. HALL, University of Colorado.

Bacteriology still suffers for the lack of a satisfactory classification and nomenclature. This is thought to be due to the failure of bacteri-

ologists to agree upon suitable criteria in the definitions of taxonomic groups. The use of habitat is particularly criticised as inapplicable to the identification of living organisms and the increased tendency to its use in recent taxonomic efforts is deplored. It is suggested that the attempt to settle taxonomic problems by committee action is likely to prove futile.

A plea is made for definitions of bacterial genera and all larger groupings upon exclusively morphologic (including tinctorial) criteria, reserving physiologic properties for the definitions of species, and excluding serologic reactions (particularly agglutination) from the definition of species.

2. Serratia indica Bergey, the Red Chromogenic Bacterium Originally isolated by Koch in India. ROBERT S. BREED AND MARGARET E. BREED, New York Agricultural Experiment Station, Geneva, N. Y.

A discussion of the history and taxonomic relationship of this organism.

This species was originally isolated by Robert Koch in 1884 from the alimentary tract of a Java ape in India, while studying the cholera organism. Cultures were sent to the laboratory in Berlin and have been preserved in various bacteriological laboratories, three strains having been secured for study. The first binomial applied to this species appears to have been *Bacillus indicus* as used by Eisenberg, 1886. Koch used *Micrococcus indicus* in 1887, a name applied to the organism by Crookshank in 1886.

Pasquale, 1891, isolated a red, chromogenic rod from soil in southern Italy which he regarded as probably identical. No other report of its isolation has been found in the literature but cultures of a red chromogen isolated in 1922 at Ithaca, N. Y., by W. A. Whiting from a poorly sterilized milk can have proved to be identical with cultures of the original.

While it is almost identical in cultural characteristics with *Serratia marcescens*, Bizio (*Monas prodigiosa* Ehrenberg), it differs from this well known organism in several distinct characters. In a gelatin stab culture, especially where no peptone or beef extract is added to the medium, it produces a brilliant, orange-red pellicle which readily settles into the liquefied gelatin in old cultures or on being shaken. *S. indica* utilizes urea more readily than *S. marcescens* and gives an abundant, pink, mucous-like growth in a liquid medium containing urea, potassium

chloride, glucose and distilled water. *S. marcescens* grows very poorly in this medium, without chromogenesis. *S. indica* is less variable in morphology than *S. marcescens*. *S. marcescens* under the same condition is highly variable, varying from coccus-like rods to distinct rods and even long filaments. The latter appear particularly well in sugar broths after acid has been produced. Intravenous inoculations of a saline suspension of *S. indica* are reported by previous investigators to kill laboratory animals more quickly than similar suspensions of *S. marcescens*. Tests on rabbits and guinea pigs have confirmed these statements.

Because this organism has been isolated so rarely it may be a rare species. Its wide distribution suggests, however, that it has ordinarily been overlooked or confused with *S. marcescens*, and that it may occur abundantly in suitable habitats.

This investigation has been made possible through a grant given by the American Association for the Advancement of Science.

3. Fermentation of Citrates. Fermentation of Citric Acid in Various Culture Media. E. G. HASTINGS, HARRIET MANSFIELD AND GEORGE HELZ, College of Agriculture, University of Wisconsin.

The fermentation of citric acid by bacteria depends on the nature of the medium. Organisms fermenting citric acid in milk do not necessarily ferment it in another organic medium, and those which ferment citric acid in an inorganic medium in which sodium citrate is the only source of carbon do not necessarily ferment it in milk. The nature of the available carbon is probably the determining factor in citric acid fermentation. Organisms found to ferment citric acid in milk are *Escherichia coli*, *Aerobacter cloacae*, *S. citrovorus* and *S. paracitrovorus*. Those which do not attack citric acid in milk are *S. lactis*, *L. casei*, *L. acidophilus*, *Aerobacter aerogenes*, casein digesters and lactose fermenting yeasts.

4. Differential Tests and Their Relation to Habitat of the Coli-aerogenes Group. S. A. KOSER, University of Illinois, Urbana, Ill.—(Lantern.)

The simpler tests used to separate intestinal *Bact. coli* from other members of the coli-aerogenes group were applied to a series of cultures isolated from known sources representing various grades of intestinal pollution. The tests employed were the methyl red and Voges-Proskauer tests and the test of citrate utilization. Over nine hundred cultures

obtained from the following sources have been subjected to these tests: (1) human and animal feces, (2) pastures, (3) cultivated fields, (4) apparently unpolluted soil, (5) raw sewage, (6) rivers, and (7) springs and other supplies classed as unpolluted. The most important points brought out were that colon organisms resembling those of intestinal origin in respect to the methyl red and Voges-Proskauer tests may be found in apparently unpolluted soil. These types may be differentiated from the true intestinal *Bact. coli* by use of the citrate test; *Bact. coli* from the intestine is citrate negative, the closely related types from soil are usually citrate positive. On the basis of this differentiation, a marked degree of correlation is found between the results of the citrate test and the degree of pollution represented by the various classes of soil and waters examined. In the work thus far, the citrate test has correlated more closely with sanitary conditions than have the other differential tests.

5. Carbon Dioxide Requirements of Bacteria. GEORGE VALLEY AND LEO F. RETTGER, Yale University, New Haven, Conn.

In attempting to answer the question, "Can bacteria initiate growth and develop in the absence of CO_2?" the following organisms (82 in all) have thus far been employed.

Coccaceae: Staph. albus and *aureus* (4 strains), *Strep. pyogenes* (1 strain), *S. lutea* (1) and *S. aurantiaca* (1), and *M. tetragenus* (1 strain).

Spirillaceae: V. cholerae (3), and *V. metchnikovi.*

Bacteriaceae: C. violaceum, E. prodigiosus (2), *Ps. pyocyanea* (4), *Ps. fluorescens* (2), liq. and non-liq., *Zopfius zenkeri, Bact. coli* (12), *Bact. aerogenes* (2 soil and 2 intestinal), *Bact. cloacae, Bact. para-typhosum,* A and B. *Bact. typhi-murium, Bact. abortivo-equinum, Bact. anatum* (2), *Bact. aertrycke* (2), *Bact. pullorum* (4), *Bact. gallinarum, Bact. enteritidis, Bact. typhosum* (11), *Bact. pneumoniae, Bact. dysenteriae* and *L. acidophilus* (7).

Bacillaceae: B. subtilis, B. cereus, B. mesentericus, B. anthracis, B. prausnitzii, B. mycoides (2), and *B. megatherium.*

All of the above organisms have failed to initiate growth in the absence of CO_2.

A study of several clostridium forms is now in progress. The evidence thus far points to the fact that spore-forming anaerobes also require CO_2 for their development.

It appears that CO_2 is necessary for all bacterial development.

In many instances, growth could be prevented by removing the CO_2

from the atmosphere with which they were supplied preliminary to and during the period of incubation. On the other hand, some of the organisms failed to develop only when both the gaseous environment and the medium were made CO_2 free.

In the second phase of the investigation it was shown that, though the bacteria employed require CO_2 for development, different organisms had definite optimum concentration limits, and that 0.3 to 1 per cent CO_2 favored higher plate counts than the amounts ordinarily present in the atmosphere (about 0.03 per cent). No added benefit resulted to the organisms from an increase of the CO_2 content of the enclosed atmosphere to 10 per cent or 20 per cent. Beyond the last-named concentration retardation of growth becomes apparent, due to increased H-ion concentration of the medium.

Different bacterial groups, and often different members of the same genus or species, vary markedly in their carbon dioxide requirements. For example, certain strains of *Proteus vulgaris* are very easily inhibited by CO_2 removal, while others are not; again *B. subtilis* fails to grow in a gaseous environment from which CO_2 is absorbed, while *B. cereus* can be prevented from developing only by the most extreme measures of CO_2 removal from the medium itself.

6. A Rapid Method for the Determination of Sugar in Bacterial Cultures. H. R. Stiles and W. H. Peterson, University of Wisconsin—(Demonstration.)

Instead of determining the titratable acid, hydrogen-ion concentration, or other indirect means of measuring sugar fermentation, it is preferable to analyze the culture for its unfermented sugar. This can be done quickly and accurately by means of the Shaffer-Hartmann micro-method as adapted to bacterial cultures.

Ten cubic centimeters of culture is placed in a 50 cc. volumetric flask, neutralized with NaOH, clarified with 1 cc. of lead subacetate (30 per cent) and without removing the precipitate is deleaded with 3 cc. of disodium phosphate (10 per cent) and the volume made up to 50 cc. The contents are shaken by inverting and in about 3 minutes the precipitate has settled and 2 cc. may be removed without filtration and the sugar determined by the micro-method of Shaffer and Hartmann. The procedure is accurate to within 10 to 20 mgm. per gram of sugar. If a somewhat less degree of accuracy is sufficient, as is usually the case in determining the question of the fermentability of the sugar, 1 cc. of culture is removed with a sterile pipette, neutralized, made to 5 cc. and

the sugar determined without clarification. This modification gives results that are 1 to 2 per cent higher than when the culture is clarified.

7. *A New Method for the Detection of Proteolysis by Bacteria.* W. C. FRAZIER, Bureau of Dairying, Dept. of Agriculture, Washington, D. C.

A new method is described by which small amounts of proteolysis of gelatine can be detected and a rough idea of the amount of this hydrolysis obtained.

Plates are poured of gelatine-agar which contains 0.4 per cent of gelatine together with salts and small amounts of peptone and beef infusion. Duplicate plates are inoculated at the center to form a giant colony and incubated at 30°C. for forty-eight hours. Then one plate is flooded with an acid solution of bichloride of mercury while the other plate is flooded with a 1 per cent solution of tannic acid. In the first a clear zone around the giant colony indicates decomposition of the gelatine as far, at least, as peptones. The width of the clear zone is indicative of the amount of proteolysis. Similarly a heavy precipitate about the giant colony in the plate flooded with tannic acid solution shows decomposition of the gelatine. There is also about the colony of an organism which breaks down gelatine to a considerable extent, a clear zone surrounded by a striking white halo. The tannic acid precipitates the gelatine and decomposition products down through some of the amino acids. By use of the two plates, then, a fair idea can be obtained of the amount of decomposition of the gelatine.

It was also found that by this method the ability of an organism to liquefy gelatine could be determined in two days. This was demonstrated with 215 cultures by comparison with the standard method for determining gelatine liquefication, and by amino acid determinations on a similar solution without agar. Even in cases where the organisms liquefy gelatine without a measurable increase in free amino acid groups, this plate method shows a decomposition of the gelatine.

8. *The Influence of Sodium Chloride on the Colorimetric Determination of pH.* L. B. PARSONS AND W. F. DOUGLAS, Laboratory of the Cudahy Packing Co., Omaha.

Electrometric comparisons have been made between Clark and Lubs buffer solutions and similar buffer solutions 1M, 2M, 3M with sodium chloride, after adding indicator and adjusting to identical colors. From these measurements the sign and magnitude of the salt error was

noted. With the following indicators: thymol blue, cresol red, phenol red, brom thymol blue, brom cresol purple, brom cresol green and brom phenol blue the errors were such that in molar salt solutions 0.2–0.3 of a pH unit (depending on the indicator) must be subtracted from the apparent value to give the actual pH. In 2M salt solutions 0.25 to 0.35, and in 3M, 0.30 to 0.43 pH must be subtracted from the apparent values. These results indicate that a satisfactory correction may be applied to solutions containing salt in the above range of concentrations to yield results which are within the accuracy of the colorimetric method.

9. The Significance of Recent Studies on Meriquinones. W. MANSFIELD CLARK, BARNETT COHEN AND H. D. GIBBS, Hygienic Laboratory, Washington, D. C.

On partial oxidation of benzidine or p-phenylene diamine or their analogues there are formed color compounds consisting of mixtures of the oxidant and reductant. These so-called meriquinones have been very extensively used in various color tests for oxidation processes in biological systems. We have shown that the first stage of oxidation resulting in the characteristic color, is a reversible process and that the complex equilibria involved can be measured by electrometric methods and defined in terms of oxidation-reduction potentials.

It is therefore possible to define numerically the intensity of oxidation required for the production of these color reactions. But it is found that the equilibria are so complex that the amount of color becomes a function of the relative concentrations of total oxidant and reductant, of the hydrion concentration of the solution, of the degree of oxidation at any given total concentration and of the dilution. Furthermore, it has been demonstrated that the condition conducive to the formation of color is also conducive to its rapid destruction.

In consequence of these quantitative findings it can be predicted that a precise quantitative analysis of the significance of color production in any given case is almost impossible; and that these color reactions will henceforth be relegated to qualitative uses. However, certain gross relationships concerning the degree of oxidation required for color production can be established. Color can be produced by oxidation of members of the phenylene diamine series at potentials comparable to those at which indophenols are partially reduced. Very appreciably higher potentials are required for oxidation of members of the benzidine series. These quantitative relations, when considered in conjunction with the fact that cell suspensions frequently oxidize the phenylene

diamines but seldom the benzidines, supports the conclusion reached by studies made with indophenols; namely that the oxidation-reduction potentials of aerated cell suspensions lie near the centre of the oxidation-reduction scale. This suggests that the cells prefer not only solutions neutral with respect to acid-base equilibria but also those neutral with respect to oxidation-reduction equilibria.

10. Sunlight as a Disinfectant. F. M. MEADER, Detroit, Mich.—(Lantern.)

In latitude 42 degrees 15 minutes, at an elevation of about 800 feet above sealevel, on a clear sunny day, direct sunlight will kill *Staphylococcus aureus* in one hour during the middle of the day. The same sunlight, passing through glass 8 mm. thick, will kill *Staphylococcus aureus* in a period of about four and one-half hours. Skylight from a northern exposure will kill *Staphylococcus aureus* in about four hours. Sunlight of the kind mentioned above on March 26, begins to be effective about 9'oclock in the morning, but has very little effect after 3 o'clock in the afternoon.

Wave lengths of sunlight which have bactericidal effect on the organisms studied are confined to the short end of the solar spectrum. When using arc lights as sources of light no bactericidal effect was found in a wave length longer than 310 millimicrons in length, during five hours' exposure. The shortest wave length in solar light at noon of April 1, at above place, was 299.2 millimicrons in length. At 7 o'clock in the evening the shortest wave length was about 310 millimicrons. Since bactericidal properties of sunlight for *Staphylococcus aureus* in direct sunlight are not apparent after 3:30 p.m., it would seem that the effective rays in sunlight must be confined to those rays which are apparent at noon, but not apparent in the late afternoon.

A summary of the theoretical considerations involved in the bactericidal properties of light is briefly reviewed from the literature.

11. Conductivity as Applied to Studies of Bacterial Metabolism. II. Parallelism between Ammonia and Conductivity in Nutrient Gelatine Cultures of Putrefactive Anaerobes. L. B. PARSONS AND W. S. STURGES, Laboratory of the Cudahy Packing Co., Omaha.

In a previous paper (Abst. of Bact., 1925, ix, 10) conductivity change was shown to be proportional to ammonia change for various strains of *C. sporogenes* and *C. flabelliferum*. An extension of these measurements has been made to gelatine cultures of *C. sporogenes*, *C. bifermentans*, *C.*

histolyticum, *C. Reading*, *C. parasporogenes*, *C. tertium*, and *C. putrificum*. Cultures were incubated in hydrogen at 20°C. and 37°C., determinations being made after two, four, six, ten and sixteen days.

For all cultures a direct proportionality was found between conductivity and ammonia. Change in Sp. Cond. (in reciprocal ohms) $\times$ 1.95×10^4 = mgm. Ammonia Nitrogen produced per 100 cc. of the medium. The difference between the calculated and observed (Folin) ammonia values was usually less than 5 per cent. The data for *C. histolyticum*, while conforming to the above equation to within 10 per cent are better satisfied by using the constant 1.87×10^4. This probably indicates a slightly different type of metabolism by this organism.

12. The Effect of Surface Tension upon the Growth of Lactobacillus acidophilus and Lactobacillus bulgaricus. W. R. Albus and George E. Holm, Bureau of Dairying, Department of Agriculture, Washington, D. C.

L. bulgaricus exhibited no growth when surface tension of the medium employed was depressed with sodium recinoleate to less than 40 dynes, while *L. acidophilus* exhibited good growth in the same medium depressed to 36 dynes. This forms a basis of differentiation for these two organisms and a plausible explanation of the inability to implant *L. bulgaricus* in the lower intestine. Fifteen strains of each organism were employed.

13. Some Relations between the Electrophoretic Potential and Other Characteristics of Variants of Pneumococci. I. S. Falk and M. A. Jacobson, University of Chicago.

Two variant strains of pneumococci obtained by Dr. F. G. Blake, Yale School of Medicine, from a type I culture grown in the presence of homologous anti-serum retain their original characteristics when grown on blood agar. We confirm the finding of early workers that these variants differ from the parent strain in virulence for white mice. Our experiments demonstrate that the electrophoretic potentials on these strains parallel their virulence. The data reported here show that the sequence as well as the absolute magnitudes change when the pneumococcus variants are washed. Our experiments suggest that electrophoretic potential is related in some way to virulence, phagocytability, agglutinability, capsule formation and other characteristics of microorganisms.

A total of 15 strains (single cell) were isolated from Blake and Trask's

A, B, and C cultures of pneumococci (A-type I; B and C are variant strains obtained from A by growth in the presence of homologous antiserum). Experiments with fourteen strains (five from A; five from B; four from C) and with parent strains demonstrate that each of the single-cell strains is indistinguishable from the parent strain from which it was derived with respect to electrophoretic potential, virulence for white mice and serum agglutination.

The isoelectric points of the pneumococcus variants B and C and of A are near pH = 3.0 and are not definitely different. The P.D. for the pneumococcus variants is correlated with the inagglutinability by acid. It is found that the higher the P.D. at pH = 6.5, the lower the pH necessary to cause spontaneous agglutination. The P.D. values for the variant types can be changed relatively by washing them. An inversion of the relative potential on variants A, B, and C is accompanied by an inversion in their agglutinability by acid. Type specific, homologous and heterologous antipneumococcus serum reduces the negative P.D.: the greater effectiveness of the homologous serum in this regard parallels its greater effectiveness in agglutinating the bacteria.

Sodium oleate, which is a specific solvent for pneumococci, dissolves the A, B, and C strains equally well. This finding is contradictory to the usual statement that virulence and solubility (bile) are parallel characteristics. The B and C variants of pneumococci are all of the S type, giving smooth colonies on nutrient or blood agar plates. From each of them we have obtained R strains by growth in the presence of specific anti-pneumococcus serum.

14. The Biologic Origin of Inagglutinability with Freshly Isolated Typhoid Cultures. RALPH R. MELLON, M.D., Highland Hospital, Rochester, New York.—(Lantern.)

It has been found possible to develop inagglutinable typhoid strains from agglutinable ones and vice versa by *in vitro* methods without the employment of immune sera, exudates or other protein substances. This change in type is thought to originate in the reorganization incident to zygospore formation that can be shown with the strains studied. Variants of all degrees of stability have been dissociated out of the plemorphic cycle. Specific metabolic changes may result in similar effects but they are sharply distinguished from genetic alterations by their purely fluctuating character. The physicochemical nature of inagglutinability has been subjected to analysis by cataphoretic methods.

15. Electrophoretic Potentials: The Virulence and Agglutinability of Pneumococci. I. S. FALK AND M. A. JACOBSON, University of Chicago.

The electrophoretic potential bears a significant relation to the stability of a bacterial suspension; and the agglutinability of bacterial suspensions is, in many instances, correlated with virulence for laboratory animals. Studies were made upon the relations between potential and virulence.

From measurements on 76 strains of pneumococci isolated from cases of lobar pneumonia (19 type I; 24 type II; 7 type III; and 26 type IV) it appears that the electrophoretic potentials are different for the several types. The highest potential (circa 11 micra per second) are uniformly found on type III organisms. The most probable sequence of decreasing potential is: III, I, II, IV. The potentials on type III organisms are sharply marked off from the potentials on the others; the potentials on Types I, II and IV organisms are not so sharply marked off. From measurements of virulence of pneumococci for white mice it appears that the sequence of decreasing virulence is: III, I, II, IV and is identical with that for decreasing potential.

Such information as is available indicates that the sequence: III, I, II, IV represents the sequence for the fatality rates in pneumococcus pneumonias of the several types. It was found that for pneumococci of types II, III and IV cultures isolated from fatal cases of acute lobar pneumonia show higher potentials than those obtained from non-fatal cases. The reverse was observed for cultures of type I.

The isoelectric points of pneumococci (types I to IV) are near pH = 3.0 and are not type specific. The electrophoretic potentials are correlated with inagglutinability by acid. The potentials are reduced by specific and by non-specific anti-sera, the greater effectiveness of the former being correlated with the greater effectiveness in causing agglutination.

Sodium oleate, which dissolves pneumococci as specifically as does bile, effects significant increases in electrophoretic potentials. Sodium oleate increases the suspension stability of pneumococci and reduces the titres of serum agglutinations.

16. The Influence of Hydrogen Ion Concentration upon the Viscosity of Bacterial Suspensions. I. S. FALK AND R. W. HARRISON, University of Chicago.

From experiments with *B. coli* it appears that the viscosity, like cer-

tain other properties, of bacterial suspensions is determined by the colloidal nature of bacteria and can be treated as though the bacteria were inert material in the colloidal state. The viscosity of bacterial suspensions is affected by the hydrogen ion concentration. Marked increases in viscosity are produced in zones of pH about the two isoelectric points of the bacteria (pH = 3–4; pH = 13–13.5) where the bacteria show marked acid and alkali agglutination. The effect of hydrogen ion concentration is slight in other pH zones. The viscosity of bacterial suspensions is a function of concentration and is higher the greater the number of bacteria per cubic centimeter of suspension. Our findings indicate that the relation between concentration and viscosity is both qualitatively and quantitatively in accord with the general equation of Arrhenius which describes the relation for colloidal suspensions of inert materials. The viscosity of bacterial suspensions, like colloidal suspensions generally, is a logarithmic function of the relative volume (Φ) occupied by the dispersed particles.

Our findings with bacteria differ from those of Loeb with gelatin and other proteins in that viscosity is *maximal* instead of *minimal* at the isoelectric points. We are led to the conclusion that the increased viscosity which becomes manifest at the isoelectric points of the bacteria is due to an apparent increase in the volume of the dispersed phase which is incidental to the agglutination of the bacteria and the occlusion of water in the clumps of agglutinated particles.

17. The Beginnings of Bacteriology in the Middle West. Dr. Wm. Trealease and Dr. E. A. Birge. (By request.)

18. The Rôle of Accessory Food Factors in the Physiology of Microorganisms. C. H. Werkman, Iowa State College.

The significance of accessory food factors in the physiology of microorganisms has been studied from three points of view: (1) Accessory food factor requirements of certain yeasts, torulae and bacteria. (2) Stimulative effects of vitamin B on rates of reproduction of microorganisms. (3) Synthesis of vitamins A and B by various microorganisms.

Contrary to general belief and conforming to the view of Fulmer and Nelson, 14 races of *Saccharomyces cervisiae* were found to grow and reproduce continuously in wholly synthetic media employing synthetic methose or succinic acid as the source of energy in Fulmer's synthetic basal medium E. *Saccharomyces ellipsoideus, Torula rosea, Torula*

liquefaciens, *Oospora lactis* and 12 species of bacteria had reproduced continuously for longer than one year, when discontinued, in wholly synthetic media employing at least three of the four following sources of energy: synthetic methose, succinic acid or distilled glycerol or seven day hot 95 per cent alcohol continuously extracted sucrose.

No addition of bios or vitamin B to the medium is required for the continuous growth and reproduction of the above organisms and if any such substance or substances are required they are metabolized by the organism.

That vitamin B exerts no stimulative effect on the rate of reproduction or the nitrogen fixed by certain microörganisms is apparent when coefficients of the rates of reproduction of microörganisms are determined and due consideration is taken of the nutritive effect of extraneous materials present in vitamin B preparations. This was found true for *Azotobacter chroococcum* and *Rhizobium leguminosarum*.

Synthesis of vitamin A or B was determined by rat experiments, both curative and preventive. Vitamin free and wholly synthetic media were used for the growth of the microörganisms. *Azotobacter chroococcum*, *Rhizobium leguminosarum* and *Oospora lactis* metabolized a substance apparently vitamin B. No such elaboration of vitamin A by the three organisms tested was found.

19. Yeast as a Substitute for Meat Extract in Culture Media. E. JOHANSON AND JEAN BROADHURST, Teachers College, New York City.

Yeast, as a substitute for meat extract, was tested in liquid and agar media. Ordinary meat extract bouillon was compared with a yeast preparation using the same ingredients (10 grams peptone, 5 grams salt to a liter of water), except that 2 cakes of (Fleischmann) yeast were substitued for the 3 grams of (Liebig) meat extract. Equal inoculations of common organisms were made into these media and the resulting growths were plated at intervals. The counts were much higher in the yeast-agar plates.

The unfiltered yeast was so cloudy that colony counting was difficult. A second yeast medium was prepared by decanting the yeast preparation already described. This yielded a much clearer agar, and the higher counts characteristic of it may be attributed to the greater degree of accuracy in computing the smaller colonies.

Typical plate counts secured from water suspension of common organisms are given in the following order: decanted yeast, unfiltered yeast and meat extract: *Serratia*, 30,600,000, 16,200,000 and 25,500,000;

enteritidis, 24,320,000, 19,840,000 and 19,840,000; *putrificus*, 50,560,000, 35,840,000 and 47,360,000; and *coli*, 198,460,000, 159,360,000 and 155,520,000. In routine examinations of drinking water, swimming pools, and milk the yeast-agar preparations proved quite satisfactory for plate counts, e.g., brook water: decanted, 19,840, meat extract, 14,720; swimming pool: decanted 32,640, meat extract, 20,480; and milk decanted, 8,000, and meat extract 3,000 per cubic centimeter.

For lactose-neutral red determination of coli the yeast preparations gave slightly better results on the whole than meat extract lactose-neutral red.

In some sixty sets of dilutions ranging from 1/100 to 1/10,000, using specific organisms and liquids of sanitary importance, such as water and milk, the yeast-agar counts have averaged 1¼ to 1½ times the meat extract counts. The substitution of yeast for meat extract might therefore be accepted for simple bacteriological work, and the addition of yeast might be expected to help in the presumptive tests made to detect organisms of the *coli* group.

20. Observations of Bacterial Inhibition and the Quantity of Inoculation. WILBERT S. SLEMMONS, Carnation Milk Products Company, Oconomowac, Wis.

I. Salt tolerance. Experiments were conducted with carefully washed suspensions of *Es. coli*, *M. albus*, and *B. mesentericus*, prepared from pure cultures of these organisms obtained from natural sources. These suspensions were standardized to bacterial amounts of 50; 5,000; 500,000; and 50,000,000 per cubic centimeter and these titres checked by means of the plate counting method. These suspensions were used in one cubic centimeter quantities to inoculate sodium chloride, nutrient bouillon of sodium chloride concentrations from 1 to 12 per cent.

The larger inoculations of the three strains used showed a greater salt tolerance for growth than the smaller inoculations. The widest variation in salt tolerance was between the 50 and 50,000 inoculations of *M. albus*, where the first was inhibited at 4 per cent and the last at 11 per cent NaCl.

Ten trials each were made in culturing the strains of various tolerances in each series into sterile NaCl bouillon of various concentration. This was done with *Es. coli* and *M. albus*. Persistence of ability to tolerate high salt concentration was exhibited by *Es. coli* and *M. albus* strains obtained by artificial selection. This persistence is a variable attribute and seemingly cannot be absolutely predicted as there were a minor number of failures.

II. Hydrogen-ion tolerance. Inoculations of the same range as those used in the above showed no marked differences in hydrogen-ion tolerance in the case of *M. albus* and *B. mesentericus* and no difference whatever in the case of *Es. coli.* The range of pH employed was from 2.4 to 6.0 with intervals of .4 of a pH. Further refinements in technique might show such a relation to exist.

21. A "Bacteriophage" Active Against a Hemolytic Streptococcus. Paul F. Clark and Alice Schiedt Clark, University of Wisconsin.

A "Phage" has been obtained active against a virulent hemolytic streptococcus. This lytic principle was secured by growing the organism with activated sludge from the Milwaukee Sewage Disposal Plant. It presents all the usual characteristics and is of interest chiefly because of the usual failure to obtain the lytic principle against any streptococcus.

22. The Rôle of the Bacteriophage in Streptococcus Infections. L. O. Dutton, Methodist Hospital, Memphis, Tenn.

In a detailed study of a large number of *Streptococcus* strains the following conclusions are indicated.

1. A large majority of the strains of *Streptococcus* isolated from the human body are mixed with the bacteriophage.

2. It was found that the bacteriophage is the factor responsible for the occurrence of large numbers of Gram negative individuals in certain cultures of the organisms, for the occurrence of certain large grotesque forms, and for the growth of the organisms in agglutinated masses in fluid cultures.

3. The variation in resistance of the organism to the lytic action of the bacteriophage may account for the variation in virulence of the bacterium, and may account for the occurrence of streptococci in the normal throat (mixed strains), the extreme resistance of certain streptococcus infections (resistant strains), and the occasional occurrence of a spontaneous recovery from a streptococcus septicemia (susceptible strains).

Experimental evidence is given in support of the above conclusion.

23. An Example of Bacterial Mutation. Roy W. Pryer, Department of Health, Detroit, Mich.—(Lantern.)

Sporococcus scarlatinae, described in previous papers from this laboratory, can be changed into a hemolytic staphylococcus by the action

of various serums which possess the power to dissolve *Sporococcus*. It is probable that a filterable stage exists between these two forms. Stabilization of mutated cultures is discussed as well as a brief summary of the properties of the two organisms.

24. Mutation-like Alterations of Certain Pathogenic Fungi. D. J. DAVIS, University of Illinois, College of Medicine.

In certain pathogenic fungi, rather sudden mutation-like alterations appear when such organisms are growing on artificial media. Such changes have been noted especially in *Sporothrica Schenkii*, and in *Penicillium brevicaule*. A description of these changes is given with a discussion of their possible significance.

25. A Genetic Inquiry into the Reason for Three Diverse Morphologic Types of Infecting Fungi in Blastomycosis Hominis. RALPH R. MELLON, M.D., Highland Hospital, Rochester, N. Y.

From a single case of blastomycosis we have isolated two of the three recognized types of parasites believed to be etiologic for the disease. The yeast type comes from the recent lesions, and the intermediate or mycelial type from the older lesions. *In vitro* experiments have shown that pure line cultures of the latter type have repeatedly yielded the mould-like or third type. This change in type has the same genetic mechanism as is operative in changes of bacterial type. An ascospore stage has been observed in the life cycle of these parasites. This so-called "perfect stage" of the mycologist permits for the first time their taxonomic allocation among the endomyces.

26. Crystal Formation in Bacterial Cultures. SARA A. SCUDDER, Evans Memorial, Boston, Mass.

The appearance of crystals, which resemble microscopically those of ammonium-magnesium phosphate, have been observed in connection with bacterial growth in a semi-solid medium. This medium consists of a beef heart hormone base with 0.5 per cent agar, 10 per cent peptone and brom thymol blue indicator, adjusted to pH 7.0. The crystals first appear in the zone of growth and later deeper in the agar. Precipitation of the crystals is enhanced by the presence of peptone and ascitic fluid, and varies with the reaction of the medium, the amount of water of condensation and the species of bacteria. No crystals form in uninoculated medium kept for months in hard glass tubes. The experimental data at hand indicate that the formation of these crystals is

the result of bacterial activity. The bacteria which are associated with the most pronounced crystal formation do not ferment the ordinary carbohydrates and intensify the alkalinity of the medium rapidly; those associated with no crystal formation ferment these carbohydrates and intensify the acidity.

27. Measuring the Growth of Bacteria with the Nephelometer. MARY WOTHERSPOON COLLEY, Madison, Wisconsin.

The nephelometer can be used to measure the degree of turbidity in bacterial cultures. The organisms should be cultured in a colorless liquid medium. Two to 10 cc. are sufficient for examination. After thorough emulsification, and sterilization, in the case of pathogenic forms, the material is ready to be placed in the nephelometer cups. The method offers a speedy and accurate means of comparing turbidity with the personal equation reduced to a minimum.

28. Variation of Temperatures within Bacteriological Incubators. A. R. WARD AND H. A. HARDING, Frederick C. Mathews Company.

The simultaneous variation of temperature in different parts of laboratory incubators was studied by means of standardized thermometers graduated to 1°F. To facilitate accurate readings, each thermometer was inserted through a perforated cork into a test tube of water. Before being placed in the incubator, the test tubes of water were heated to 100°F.

The temperature of nine locations within the incubator chamber were studied, as follows: Left, center and right of two shelves, and similar locations 6 inches above top shelf. Variations of 0.5° to 6.5° Fahrenheit were observed among nine incubators.

AGRICULTURAL AND INDUSTRIAL BACTERIOLOGY

1. An Experimental Study of the Organisms Concerned in the Commercial Production of Acetone and Butyl Alcohol by the Biological Method. ELLIOTT R. WEYER AND LEO F. RETTGER, Yale University.

This organism which is of extreme practical significance has by some been incorrectly called *Granulobacter pectinovorum*. It has also gone by the names *"Bacillus butylicus," "Bacterium butylicum,"* etc. It would seem as if re-baptism is highly desirable. It is a Gram-positive anaerobic organism which produces spores.

In the present investigation isolations were made from corn, barley,

soil and the stomach contents of clams. Attempts to isolate the organism from other cereals and from feces have not been successful.

Well developed and sharply defined surface colonies were obtained on malt extract agar plates incubated in culture jars after complete exhaustion of the contained air. A more practical method, however, is one in which the air is excluded by inverting the bottom of an ordinary Petri dish in a Petri dish lid containing the inoculated malt extract agar.

All strains are carried in 5 per cent corn mash and transferred every two weeks, and until abundant sporulation has taken place. Viability and ability to produce acetone and butyl alcohol in corn mash is greatly enhanced by making transfers from the old cultures after they have been heated at 100° for forty-five seconds. In this way the production of acetone and alcohol was increased in two years 20 per cent or over.

Inoculating the mash with *Staph. aureus* 12 hours previous to the addition of the spore-containing inoculum results in a good growth and rapid and vigorous butyl fermentation without the use of a vacuum pump. The high acidity produced kills off the *Staph. aureus* within a few hours.

Complex nitrogenous substances are necessary. Protein-containing media and ordinary peptone gave good growths.

2. A Group of Non-Spore-Forming Soil Bacteria of Peculiar Morphology. H. J. CONN, N. Y. Agric. Exper. Station, Geneva, N. Y.

It has been observed for some time that of the bacteria in soil growing on gelatin plates about 50 to 80 per cent are non-spore-forming bacteria producing punctiform colonies in gelatin or agar. They have been called by the author "slow growers," or more recently by a more descriptive designation "punctiform-colony-formers." A study of these organisms has shown them to be very diverse both in morphology and physiology.

Special interest has been aroused by one particular subdivision of this group of organisms. The bacteria comprising this smaller group are called by the author "coccus-forming rods." The character by which this group is distinguished is that they appear for one or two days upon a fresh substratum as ordinary small rods, but after that change in morphology and are indistinguishable from micrococci.

3. The Urease Content of Nitrogen Fixing Bacteria. J. ARLINGTON ANDERSON, Biochemical Department, University of Stockholm and the Bacteriology Department, Central Swedish Agricultural Experiment Station.

Stable enzyme preparations of *B. radicicola* and *Azotobacter chroococcum* were made and their urease content quantitatively determined. The values obtained were very small, being of the order of magnitude of preparations from other soil bacteria, excepting urea fermenters. No difference was found between rod and bacteroid forms of *B. radicicola*. The author concludes that while it is not impossible it appears very improbable that urease performs a rôle in nitrogen fixation.

4. The Root-Nodule Organism of Dalea Alopecuroides (Wood's Clover). A. L. WHITING, University of Wisconsin.

The bacteria from the root nodules of *Dalea alopecuroides* have been isolated in pure culture and their cultural characteristics studied. These organisms grow rapidly on mannitol agar, forming a slime which is opaque and resembles somewhat the growth of the nodule bacteria of lupines.

From the results of many cross-inoculation tests, it has been found that this organism does not form nodules on any of the common legumes and hence it has been assigned to a separate group. Because of the general characteristics of the organism, it belongs to the bacillus group of the nodule bacteria, *Bacillus radicicola*.

5. A Possible Explanation for the Specificity of Cross-Inoculation Groups in the Legume Family. I. L. BALDWIN, University of Wisconsin.

The seed protein relationships existing between certain members of the legume family has been investigated by means of serological reactions, using the precipitin and anaphylaxis reactions. The results of this investigation indicate that the legumes belonging to the same cross-inoculation groups possess similar seed proteins. The legumes belonging to different cross-inoculation groups appear to have proteins of decidedly different characteristics.

6. Seasonal Variations in the Ammonia and Nitrate Content of Lake Waters and Their Relation to Certain Types of Bacteria. B. P. DOMOGALLA, Biochemist, City of Madison.

The seasonal variation in the ammonia, nitrate, and oxygen content of certain of the inland lakes of Wisconsin was studied for two and one-half years. Variations in these substances was found to be due almost entirely to a seasonal fluctuation in the bacterial flora of the lakes. Somewhat similar differences were noted in the surface waters, but were more marked in the bottom waters.

The ammonia and nitrate content reach a maxium about March, at a time when the ammonifiers and nitrifiers are most active. During mid-summer and fall nitrate reduction becomes more active than at other seasons of the year and hence the disappearance of nitrates.

7. Oxidation of Sugar on Trickling Filters. MAX LEVINE, G. W. BURKE AND J. H. WATKINS, Iowa State College.

A 0.5 per cent skim milk to which various concentrations of lactose were added was passed through an experimental lath trickling filter. The filter was constructed so that samples could be taken at every foot of depth. Reducing sugar was determined gravimetrically.

With an initial content of 321 p.p.m. of lactose, the effluent from the first one foot of the filter contained only a trace. With 1100 p.p.m. of lactose the effluent from the first foot showed 176 p.p.m. and that from the second foot none. Beginning with 3456 p.p.m. of lactose the quantities remaining after various depths of filtration were as follows: 1 foot, 1480; 2 feet, 855; 3 feet, 360; 4 feet, 183; 5 feet, 91; 6 feet, none.

Members of the colon group of bacteria are important in the oxidation of acids produced by the initial fermentation of lactose.

8. Some Observations on the Purification of Creamery Wastes. MAX LEVINE, G. W. BURKE AND J. H. WATKINS, Iowa State College.

The disposal of creamery wastes has become a vexing question in many states. The commonly employed septic or Imhoff tanks followed by intermittent sand or trickling filters have not proven satisfactory.

Carefully collected samples (composites of fifteen-minute samples taken throughout the day) at a number of representative Iowa creameries, indicated that the concentration of wastes varied from about 1 to 5 per cent skim milk. The oxygen demand of these wastes was 10 to 50 times that of domestic sewage.

Anaerobic treatment of creamery wastes is considered theoretically wrong because of development of acidity which prevents proteolysis, and interferes with nitrification in the filters.

A reaction of pH 5.5 was found to markedly inhibit proteolysis of gelatin and casein, and at pH 5.0 many of the proteolytic bacteria isolated from creamery wastes were killed.

A concentration of 1 to 1.5 per cent skim milk is sufficient to develop these detrimental acid reactions.

The acid producing constituents may be readily removed by oxidation on suitable trickling filters. Concentrations as high as 7 per cent skim milk yielded effluents which did not become acid in anaerobic storage.

Stable effluents were obtained with 1.5 per cent skim milk, after filtration through 6 feet of cinders at a rate of 800,000 gallons per acre per day. Oxygen demands of 600 to 800 p.p.m. were reduced to 15 to 40 p.p.m. Stronger wastes did not yield stable effluents.

9. Acidity in Relation to Bacterial Changes Induced by Freezing. JEAN BROADHURST AND LAURA GILPIN, Teachers College, New York City.

Since frozen foods, such as ice cream, have a wide range in acidity, (commercial and home ice creams examined ranging from pH 7.8 for vanilla ice cream to 3.2 for lemon and orange ices), the differences in acidity might be expected to show some relationship to freezing effects, and throw some light upon the varying results obtained in recent milk and ice cream investigations.

Milk samples with varying initial counts (30,000 to 2,740,000 per cubic centimeter) were halved, and lactic or acetic acid was added to one of each of these paired samples, changing the acidity from pH 7.8 to 6.8, 7.5 to 6.4, 7.2 to 6.0, 7.2 to 4.8, etc. These paired samples were frozen (exposed for 24 hours) in the water-cube pans of an electric refrigerator, bacterial determinations (plated dilutions) being made before and after freezing.

Bacterial determination made *immediately* after adding the acid showed an increase in practically all of the treated samples, due, probably, to the "cutting" effect of the acid upon the bacterial masses. *Following freezing*, however, the acidified samples showed a decided drop in bacterial count.

From these results it is evident that the bacterial content of a given frozen milk product may be materially decreased by increasing its initial acidity.

Another conclusion, which may be of far-reaching importance, seems warranted by the *apparent* increase obtained immediately upon the addition of acid. Standard acidities are probably necessary for securing comparable determinations of the bacterial content of milk, ice cream and perhaps any substances including water, where the developing colonies are counted. For convenience, as well as for the effect upon the bacterial masses, such acidity standards should be slightly beyond the highest acidity ordinarily occurring in a given substance, e.g., more acid than 3.2 for fruit ices or more acid than 5.2 for ice creams.

10. Thermophilic Bacteria and Milk Pasteurization. H. A. HARDING AND A. R. WARD, Frederick C. Mathews Company.

Bacteria capable of growing at high temperatures have long been known but an appreciation of the constant presence of such germs in all milk is not yet widely spread.

Spore bearing rods seem most common but it has not been demonstrated that rods are responsible for the outbreaks in pasteurized milk.

The occurrence of large numbers of thermophilic germs in the output of a given plant does not appear to depend upon any recognized factors. The outbreaks occur irregularly and at all seasons of the year. Presumably such outbreaks are of fairly frequent occurrence in practically all supplies of pasteurized milk.

11. Thermophilic Bacteria from Milk. F. W. TANNER AND H. G. HARDING, University of Illinois.

Thermophilic bacteria, though not numerous, have been demonstrated in every sample of milk obtained after the milk had left the barn. From milk seventy-three cultures of thermophilic bacteria were isolated at 55C.° on plain nutrient agar, pH 6.8. It is probable that thermophiles, which do not grow on this medium, are present in milk. All the cultures were motile, Gram positive, spore-forming rods which grew well at pasteurizing temperatures, 62.5°C. (145°F.). Several of the cultures were strict thermophiles; while others were facultative thermophiles, some even growing at 20°C. Most were facultative anaerobes but many were strict aerobes. Most of the cultures digested starch, produced acid and no gas from glucose and saccharose, and did not produce acid from lactose.

12. Thermotolerant Saccharophilic Organisms as a Cause of "Pin-Point" Colonies in the Bacteriological Analysis of Ice Cream. A. C. FAY, Kansas Experiment Station, Manhattan, Kansas.

In making counts of ice cream, on standard plain agar, large numbers of "pin-point" colonies on the low dilution plates (1:100) were noted, but not in proportionate numbers or none in higher dilutions (1:1000).

Re-plating of samples of sucrose agar revealed the "pin-point" colonies in all dilutions and in proportionate numbers. Furthermore, re-plating on plain agar, containing in the higher dilution plates 0.001 cc. of sterilized ice cream, which supplied the same amount of sugar as was present in the lower dilution, resulted in the growth of the "pin-point" colonies in all dilutions.

With a few exceptions all pure cultures isolated from these "pin-

point" colonies would withstand 3 or more pasteurizations at 145° for thirty minutes without decrease in number. All cultures were thermotolerant but not highly thermophilic since none grew above 45°C.

They were either Gram positive, non-spore-forming short rods resembling *S. lactis* or somewhat longer rods irregular in morphology and in longer chains. Biochemical studies were made.

13. Some Facts About Streptococcus Lactis and the Litmus Milk Reaction. KARL J. DEMETER, Suedd. Forschungsanstalt fuer Milchwirtschaft Weihenstephan and Department of Dairy Industry, Cornell University, Ithaca, N. Y.

In the litmus milk reaction the curdling power of *Str. lactis* is influenced by different milks. Skimmilk has been proved to be more easily curdled than whole milk, and milk, whose fat is extracted with ether, much easier than skimmilk. Only such strains as had been in artificial culture longer than nine months showed a quicker souring in whole than in skimmilk. But there are no sure signs that the fat content is responsible for that difference.

The reddening of the white reduced curd proceeds more rapidly, the shorter the time required to curdle. The velocity with which that takes place is more closely correlated with the lack of fat than is the case with the curdling power. There exists great variability also between the different strains, so that the penetration of the O from the air cannot alone be the reason of the oxidation of the leukobasic litmus.

There is also much difference in the curdling time and development of the red curd, if the milk is incubated either at 30° or at 37°C. The curdling power is distinctly greater at blood temperature than at 30°. That fact shows clearly that there seems to be a close relationship of *Str. lactis* with *Str. faecalis*, as is suggested by former authors.

14. The Application of Growth Curves to a Study of the Bacteriology of Cheese. W. R. ALBUS, Bureau of Dairying, Department of Agriculture, Washington, D. C.

Two factors contributing to the cause of abnormal gassy fermentations in Swiss cheese are the physiological condition of the cells of the gas producing organism, whether aerobic or anaerobic, at the time the cheese is made and the physiological condition of the cells of the organism used to suppress the abnormal fermentations, i.e., *L. bulgaricus*.

By the application of the principles of growth curves it was found possible markedly to influence the ability of the causative organism to

produce abnormal gassy fermentations in Swiss cheese, to increase the efficiency of the bulgaricus starter in its ability to suppress abnormal gassy fermentations in Swiss cheese and to increase the efficiency of the lactic starter in the manufacture of Cheddar cheese.

15. The Destructive Action of Certain Bacteria on The Virus of Tobacco Mosaic. MAURICE MULVANIA, College of Agriculture, University of Wisconsin.

Pure cultures of a number of bacteria were grown in ordinary bouillon to which had been added definite amounts of filtrates of juice from mosaiced tobacco plants. The tubes containing the bouillon-filtrate combination were allowed to stand for several days to insure bacterial sterility before those to be tested were introduced.

It was found that some of the bacteria destroyed the infective property of the virus when grown in the above cultures while others did not. There appears to be no characteristic common to those which destroyed the virus which was not at the same time applicable to some of those which were harmless to it. A certain cellulose-fermenting thermophilic organism proved to be very injurious to the virus. Likewise such bacteria as *B. proteus* and *B. aerogenes* almost completely inactivated the infective agent of tobacco mosaic. On the other hand, such bacteria as *Sar. lutea*, *B. anthracoides* and *B. Hartlibii* were slightly, if at all, destructive to the virus.

16. Notes on Yeasts in Carbonated Beverages. CHAS. E. MCKELVEY, Introduced by Max Levine, Iowa State College.

In a study of deterioration of carbonated beverages, yeasts were found to be the most frequent causative agents. Of over 1500 samples examined during a period of two years about 85 per cent had spoiled as a result of yeast growth.

Of 132 samples of sugar obtained from 125 bottling plants in 28 states 62 were found to contain yeasts (47 per cent).

Carrot agar, saturated with calcium sulfate, was found to be suitable for the development of yeast spores and the production of large quantities for experimental work.

Yeast spores obtained from carrot calcium sulfate agar were tested as to their resistance to heat in syrups of various concentrations of cane sugar. In general the higher the concentration of sugar the longer was the time required to kill the yeasts under examination.

At 70°C., working with a mixture of the spores of 27 yeasts isolated

from carbonated beverages, inoculated into sterile syrups of different densities, the time required to kill was 24° Baume, two minutes; 27° Baume, two minutes; 30° Baume, three minutes; 33° Baume, five minutes; 36° Baume, five minutes. At 36° Baume the liquid is almost a saturated sucrose syrup at room temperature.

17. The Effect of Sodium Benzoate Upon Certain Yeasts. FRED W. TANNER AND LUELLA B. STRAUCH, University of Illinois.

The data collected under the conditions of these experiments indicate a marked difference in resistance of pure yeasts to sodium benzoate. On glucose agar and in glucose broth even in the presence of 0.4 per cent of sodium benzoate, growth was appreciable. *Torula communis* did not grow and one strain of *Sacch. ellipsoideus* was inhibited after the concentration of the benzoate in the broth had reached 0.15 per cent. Another strain of *Sacch. ellipsoideus* showed a greater resistance; it grew in the presence of even 1 per cent, but the growth was slower and less abundant. The fungi used seemed to be able to tolerate the sodium benzoate better when growing on a solid medium containing it than when growing in a liquid medium. Two of the strains of budding fungi, *Mycoderma vini* and a pure culture from vinegar (No. 18) were able to grow apparently as well in the presence of 1 per cent of sodium benzoate as in the controls.

Sterile apple juice was almost as good a medium for the yeasts as the sterile glucose broth. However, when sodum benzoate was added the growth was markedly inhibited since none of the fungi grew after 0.01 per cent had been added. The same was also true for the amount of alcohol formed.

Attempts were also made to study the preserving effect of sodium benzoate in catsup. No growth of the yeast could be secured even in control tubes containing no sodium benzoate. The most important facts brought out are the marked differences among yeasts in resistance to sodium benzoate; and the great influence exerted by the chemical constitution of the material to be preserved.

18. The Rôle of Starch in The Cracking of Chocolate Creams. BURTON G. PHILBRICK, Skinner, Sherman and Esselen, Inc. Boston, Mass.

Examination of cracked or blown chocolate creams has shown the presence of a facultative anaerobic bacillus, capable of fermenting glucose. The bacillus probably enters through some one of the ingredients but it is difficult to establish which is the infecting material, as the spoilage is often not apparent until the materials have been consumed in the

factory process. On the other hand examination of the moulding starch will generally show the presence of the organism, since the starch acts as a secondary infecting agent long after the original has been eliminated by use. The infected starch should be discarded to effect a complete and final elimination of the infection.

19. Sugar as a Source of Anaerobes Causing Explosion of Chocolate Candies. JOHN WEINZIRL, University of Washington, Seattle, Wash.

Thirty-three samples of sugar of various kinds were analyzed and the relative number of anaerobes present determined. Anaerobes were found in 85 per cent of the samples tested. The different kinds of sugar showed about the same number of anaerobes, but the number of samples of each kind was too few for drawing positive conclusions. Five types of organisms were found, as follows: *Clost. sporogenes, Clost. putrificum, Clost. aerofetidum,* and two not identified.

COMPARATIVE PATHOLOGY AND IMMUNOLOGY

1. Studies on the Etiology of Distemper in Foxes. R. G. GREEN AND H. O. HALVORSON, Medical School, University of Minnesota.

Epidemics have occurred on silver fox ranches entailing a mortality as high as 70 per cent. The most common lesions have been pneumonia, intestinal lesions, and an enlarged spleen, with a general toxic effect on all organs.

In some epidemics pneumonia occurred only occasionally, with the upper respiratory tract normal. The blood, abdominal organs and central nervous system were found to contain the virus. Filtration experiments showed the infective agent to be non-filterable. The most constant bacteriological finding was the presence of members of the *Salmonella* group. They appeared in pure culture in most ranch foxes. They occurred in pure culture almost always when the disease was transmitted by injection of infective material.

Pure cultures of *Salmonella* obtained from foxes were pathogenic and would cause death in from twelve days to thirty days producing the typical disease. After injection the organism can be recovered in pure culture at autopsy.

2. Changes in Intestinal Flora of Rats on a Calcium Deficient Diet. RACHAEL E. HOFFSTADT AND SYLVIA J. JOHNSON, Department of Bacteriology, University of Washington.

Twenty-six hooded and white rats were fed on a diet deficient in calcium and ten on the same diet with calcium supplied. Fecal examinations were made at intervals of seven days for total bacterial count and types of aerobic and anaerobic organisms present. Autopsies at death were performed on all animals. It was found that the calcium deficient diet caused a definite constipation as shown by physical symptoms and a lowered bacterial count. This may or may not have coincided with the appearance of rickets depending upon the initial physical condition of the animals. The types of organisms present were not different in either group of rats. No gross pathological changes were noted, except an apparent thinning of the wall of the small intestine.

3. L. acidophilus in Epilepsy. N. KOPELOFF, M. P. LONERGAN AND P. BEERMAN, Psychiatric Institute, Ward's Island, N. Y.

Owing to the importance of the gastro-intestinal tract in epilepsy, the investigation of *L. acidophilus* in this condition was undertaken. A series of 15 epileptics were carefully observed for one month, following which 12 of these (the other 3 had only one convulsion per month or less) were given one liter of sterile milk for forty-two days. The methods employed here have been described in "Lactobacillus Acidophilus" (The Williams & Wilkins Co., Baltimore, 1926).

Ingestion of sterile milk did not influence the number of convulsions. Neither did the ingestion of 60 grams of kaolin daily for one month. But when the same quantity of *L. acidophilus* milk, one liter, reinforced with 300 grams of lactose, was administered for two months, there was a striking reduction in the number of convulsions in 5 patients. A slight reduction was also noted in 4 other patients. Three patients remained the same and one showed a slight increase in the number of convulsions.

In the two-month period following administration of *L. acidophilus* milk all the patients (with one exception) had an increased number of convulsions. It appears, therefore, that the treatment was not sufficiently prolonged to confer permanent benefit. However, the results are encouraging enough to continue the treatment on a larger series of patients. This is now being done as well as the repetition of the above experiments on the same patients.

4. The Influence of L. Acidophilus on the Colon-Aerogenes Group in the Intestine. NICHOLAS KOPELOFF AND PHILIP BEERMAN, Psychiatric Institute, Ward's Island, N. Y.

L. acidophilus has been shown to reduce the *relative* numbers of the colon-aerogenes group in the intestine, but the question of *actual* reduction is a perplexing one. The present study was carried out on a series of 36 epileptic patients under daily observation. Fecal examinations were made weekly for seven months, comprising the periods before treatment, administration of sterile milk or kaolin, *L. acidophilus* milk, and after treatment. Platings were made on whey agar and the Ayers and Rupp synthetic medium for the enumeration of the colon-aerogenes group. The fecal specimens were dried and proper calculation for moisture made.

The results proved to be extremely variable, since the patients were on the routine hospital diet. In fact it was impossible to arrive at any clear-cut conclusions because of the large experimental error. In general it may be said that in about one-half the instances an actual reduction in numbers of the colon-aerogenes group was obtained, when averages were computed. The problem, therefore, cannot as yet be regarded as solved.

5. Experimental Bacillus pyocaneus Keratitis. EDNA JACKSON AND F. W. HARTMAN, Henry Ford Hospital, Detroit, Mich.

The virulent strain of *B. pyocaneus* used in this work was isolated from a group of industrial cases of keratitis.

The bacillus was isolated from only seven of eighteen cases all clinically similar. In some *B. pyocaneus* was isolated along with *Staphylococcus aureus* and only the reproduction of the rapidly spreading digesting lesions in rabbits convinced us that the former was the etiological factor. Injection of the culture between the layers of the cornea was the only method of infection which reproduced the disease.

Since the only effective treatment of the lesions was the destruction with the cautery, it seemed worth while to investigate the possibility of active and passive immunity in the rabbit. Results of the serum treatment of the disease in rabbits will be reported.

6. Cladothrix Isolated from the Genito-Urinary Tract. SARA A. SCUDDER AND DAVID L. BELDING, Evans Memorial, Boston, Mass.

Three strains of *Cladothrix*, as yet unindentified have been isolated from the genito-urinary tract. Strain A was obtained from the cervix of a child. Strains B and C were obtained in almost pure culture from the prostatic secretion of patients who had clinical signs of chronic urethritis with no clinical or laboratory evidence of gonorrhea. When

first isolated, all three strains morphologically and culturally resembled long-chained streptococci, but under favorable conditions of growth, they showed a marked pleomorphism, varying in form from streptococcus-like chains to long wavy filaments with transverse constrictions and branching. The filaments, which varied from 0.6 to 1.7 microns in diameter and 2.2 to over 100 microns in length showed definite protoplasmic differentiation when stained by an alkaline modification of Gram's method. Lancet-shaped diplobacilli were present in the young cultures and large globoid bodies were observed frequently in the old cultures. The strains grew well in broth which had a reaction from pH 6.8 to pH 8.0 the tangled threads forming fluffy masses which were either suspended in the fluid or adherent to the sides of the tube. In broth which had a reaction over pH 7.6 the long strands interlaced in a weblike formation and if undisturbed, remained suspended from the meniscus. The three strains were similar in carbohydrate fermentation, but different in the quantity and rapidity of acid production. In contrast to the other two, Strain C had shorter chains, produced greater cloudiness in broth, formed acid more rapidly, and presented minor serologic differences.

7. *A Simple Modification of the Brilliant Green Method of Isolating Bacterium Typhosum from Feces.* Morris L. Rakieten and Leo. F. Rettger, Yale University.

The method is essentially that of Browning, Gilmour and Mackie, and used by Robinson and Rettger in their earlier work. The only real departure is the use of a buffer in the brilliant green peptone solution which constitutes the preliminary enrichment medium. The stock buffer employed was a mixture of 7.5 grams each of KH_2PO_4 and K_2HPO_4 in 155 cc. of distilled water. Of this solution 0.4 cc. was added to 5 cc. peptone solution, or 1 per cent. The buffered brilliant green peptone tubes had a hydrogen-ion concentration of pH 6.5. The amount of buffer used was sufficient to keep the pH below 7.0 and thus prevent the removal of the brilliant green from solution during the incubation period. Platings were made from the enrichment tubes after from eighteen to twenty-four hours incubation at 37°C. on the modified Endo's medium of Robinson and Rettger.

This method was employed on more than 200 samples of feces which were artificially infected with *Bacterium typhosum*, with consistent results. In addition, it was used by the authors in the isolation of *Bacterium typhosum* at the Lenox Hill Hospital, New York, during the

typhoid epidemic in the fall of 1924. In the bacteriological examination of the stools of 14 clinical cases isolations were obtained in 70 per cent of the cases. Seven different brands of brilliant green were employed. Grübler's and one of the American brands were the most satisfactory.

8. The Use of Glycerin in Brilliant Green Bile for the Isolation of B. Typhosus from Feces. SOPHIE A. DEHLER AND LEON C. HAVENS, State Board of Health, Montgomery, Alabama.

Brilliant green bile does not inhibit bacterial growth in some specimens of feces, thus making difficult the isolation of *B. typhosus*, if present, in such specimens. It was found that the addition of glycerin prevents, to a greater extent, the growth of all fecal bacteria. Various percentages (10 to 30) of glycerin in bile were tried. Difco bile dissolved in 10 and 15 per cent glycerin in distilled water, with various concentrations of brilliant green, did not cause appreciably greater inhibition than the brilliant green bile without glycerin. Twenty and thirty per cent glycerin caused marked inhibition of the fecal flora. Further experiments showed that 20 per cent glycerin bile containing brilliant green in the concentration of 1:10,000 did not inhibit *B. typhosus*, but prevented overgrowth of fecal organisms. Plates streaked with a loopful of this medium containing feces artificially inoculated with small numbers of typhoid bacilli were rarely overcrowded, and frequently typhoid colonies predominated.

The advantage of this medium is the increased ease and accuracy in examining the plates, due to the marked inhibition of the fecal bacteria and the increase in tne numbers of typhoid bacilli.

9. Immunological Studies in Tuberculosis. IV. Concerning the Resistance to Infection of Animals Sensitized with Killed Tubercle Bacilli. S. A. PETROFF AND F. W. STEWART, Research and Clinical Laboratory, Trudeau Sanitarium.

Guinea pigs sensitized with dead tubercle bacilli were rendered skin-sensitive to tuberculin. At the height of the tuberculin reaction they were inoculated with living tubercle bacilli. Such animals showed an increased resistance over normal controls inoculated at the same time and kept under the same conditions.

10. A New Method for Suspending and Counting Living Tubercle Bacilli. FREDERIC B. JENNINGS, JR., Research and Clinical Laboratory, Trudeau Sanitarium.

Living organisms are suspended in buffer solution, filtered through paper, and counted in a bacterial counting chamber. Such suspensions are free from clumps, do not agglutinate spontaneously, and the counting is simple and accurate.

11. A Study of the Blood Groups Among the American Indians. CLARA NIGG, Department of Bacteriology, University of Kansas.

A study of 800 American Indians shows an incidence of Group I Blood Grouping of 70 to 72 per cent.

12. Data Bearing on the Question of the Size of the Antibody Molecule and the Amount of Antibody Substance Present in an Immune Serum. F. M. HUNTOON, Research Laboratory, H. K. Mulford Co., Glenolden, Pa.

Calculations based on the total solids and nitrogen content of antibody solutions not only enable the placing of a limit to the amount of antibody substance present but in the case of agglutinins establishes an outside limit of the size of the molecule of 31 millimicrons.

13. Precipitation with Fractions of Syphilitic Serum. PEARL L. KENDRICK AND R. L. KAHN, Department of Health, Lansing, Michigan.

An attempt was made to determine what fraction of syphilitic serum contained the reacting substances responsible for precipitation. The serum fractions were obtained by various degrees of saturation with ammonium sulfate. The precipitin reacting substances were determined quantitatively by means of the Kahn test.

Preliminary experiments indicated that the globulin fraction of syphilitic serum obtained after 33.3 per cent saturation with ammonium sulfate contained less of the precipitin substances than the original serum; the fraction obtained after 40 per cent saturation contained almost the same amount and the 50 per cent fraction, the same amount as the serum.

Comparative determinations of the precipitin substances were then made with the fractions obtained after 50 per cent, 75 per cent, 100 per cent ammonium sulfate saturation and the original serum. It was observed that all these fractions as well as the original serum were of approximately the same potency, indicating that the albumin fraction was free from precipitin substances. This observation was corroborated by negative findings given by the albumin fraction obtained after com-

pletely saturating the supernatant fluid of the 50 per cent globulin fraction.

These findings indicate that the globulin fraction of syphilitic serum obtained after 50 per cent saturation with ammonium sulfate contains all the reacting substances responsible for positive precipitations as determined by the Kahn test.

14. The Effect of Certain Substances on the Precipitin Reaction. CORNELIA M. DOWNS AND KENNETH GOODNER, Dept. of Bacteriology and Immunology, University of Kansas, Lawrence, Kan.

In the precipitin-preciptinogen reaction the presence of foreign protein does not increase the prozone nor inhibit the reaction.

The precipitate dissolves in weak carbonate solutions. Preciptinogen is present in the fluid but no precipitin is detectable until the solution is neutralized. The precipitate is completely dissolved in 40 per cent glucose with the appearance of both precipitin and precipitate in the solution.

15. A Rapid Method for the Macroscopic Agglutination Test. ARLYLE NOBLE, Medical Research Department, Park Davis and Company, Detroit, Mich.

Using concentrated antigen, with small quantities of serum and antigen, shaking is substituted for incubation. Readings are made in from two to twenty minutes.

16. Placental Transmission of Foreign Protein. FRANCES E. HOLFORD, University of Wisconsin.

The placental transmission of foreign protein is under investigation. By precipitin tests on the serum of the new-born, transmission of crystalline egg albumin injected subcutaneously near term has been demonstrated both in normal and in highly immunized rabbits.

17. Experiments on the Solubility of Pneumococci in Bile and in Sodium Oleate Solutions. I. S. FALK AND S. Y. YANG, University of Chicago.

We have found that washed suspensions of pneumococci in distilled water are usually bile soluble. An occasional preparation is refractory to the solvent action of the bile. The chlorides with monovalent cations (Na, K, NH_4, Li) in relatively low concentrations inhibit the dissolution of washed pneumococci by bile; in higher concentrations these chlorides

do not inhibit, indeed, may accelerate the dissolution of the bacteria. Chlorides with divalent cations (Ca, Ba) behave quite differently. They are found to inhibit bile solution of pneumococci more effectively in high than in low concentrations. Of the anion series tested, NaOH and Na_3PO_4 are cytolytic to pneumococci; Na_2HPO_4, NaH_2PO_4, Na_2SO_4 and $NaNO_2$ are not cytolytic. Cytolysis by NaOH and by Na_3PO_4 appears to be a function of the hydroxyl ion concentration. Peptone, gelatin and ovalbumin appear to inhibit cytolysis by bile in the same manner as $CaCl_2$ and $BaCl_2$. The inhibitory action increases with concentration. The differences in the behavior of chlorides of mono- and di-valent cations recorded here appear to be in harmony with the usual findings in general physiology, whether effects upon animals and animal tissues, upoh chlorophyll-bearing plants, bacteria or upon non-living substances are studied.

In the concentrations in which it is active (1.25 per cent and higher) sodium oleate dissolves only pneumococci of those strains which are also dissolved by bile. In effective concentrations, sodium oleate, in aqueous solutions, dissolves washed pneumococci resuspended in distilled water; it does not dissolve pneumococci which have been resuspended in 0.85 per cent NaCl solution or in the supernatant of the culture fluid from which they had been separated by centrifugalization. The bacteriolysis of washed pneumococci by sodium oleate, sodium hydroxide, or by sodium phosphate (tribasic) is as specific as lysis by bile. Suspensions of bile insoluble strains of pneumococci, of strains of *Streptococcus hemolyticus*, *Str. viridans*, *Str. lacticus*, *M. catarrhalis*, *Sarc. lutea*, *Staph. albus* and if *Staph. aureus* are not dissolved by these reagents.

18. Action of Ultra Violet Light upon the Antigenic Property of P. avicida. L. D. Bushnell, Kansas Agricultural Experiment Station, Manhattan, Kansas.

Ultra violet light has a deleterious effect upon the antigenic properties of *P. avicida* as compared to the effect of heat killed cultures.

19. The Fate of Bact. Typhosum and Other Organisms in a Segregated Vein and in the General Circulation of the Normal and Immunized Rabbit. Ralph G. Mills and Gail M. Dack, Department of Hygiene and Bacteriology, University of Chicago, Chicago, Ill.

A marked reduction of the bacterial count followed the introduction of organisms both in the segregated vein and the general circulation. The

rate of reduction differed however. It is suggested than an additional factor is involved in the destruction in the general circulation possibly the phagocytic power of fixed tissue cells.

20. Notes on the Bacteriology of Two Cases of Undulant Fever in Man. CHARLES M. CARPENTER, Department of Pathology and Bacteriology, Cornell University, N. Y. State Veterinary College.

Discussion of the occurrence of *Brucella abortus* in man, with a bacteriological report of two cases of Undulant Fever.

21. Is Bact. abortus (Bang) Pathogenic for Humans? I. FORREST HUDDLESON, Michigan Agricultural Experiment Station, Michigan State College.

The writer reports three cases of recurrent fever in young men during the past year and evidence which incriminates *Bact. abortus* as being the causative agent.

In the first case, no hemo or urine culture was made, but the agglutinin titer of the blood serum was found to be 1:400 one year after the appearance of clinical symptoms. This case resembled the ambulatory type of recurrent fever.

The second case, clinically resembled the typical type of recurrent fever. The blood serum, one week after the appearance of clinical symptoms, showed an agglutinin titer of 1:1000 for both *Bact. abortus* and *Bact. melitensis.* An organism was cultivated from the blood in atmosphere containing 10 per cent CO_2 which resembled *Bact. abortus* culturally and serologically.

The third case resembled the ambulatory type of the disease. The blood serum shows an agglutinin titer of 1:200 and positive culture. All three of the young men have worked with *Bact. abortus* in the laboratory. Case No. 3 has also worked with *Bact. melitensis.*

Cases No. 2 and 3 had been drinking raw milk for some time from the same source. The raw milk contained *Bact. abortus* in large numbers.

22. A Method of Increasing the Virulence of Cultures of Clostridium chauvei by the Use of Ferric Salts. JOSEPH P. SCOTT, Kansas Agricultural Experiment Station, Manhattan, Kansas. (Read by Dr. L. D. Bushnell.)

The addition of ferric sulphate or ferric chlorid to Hall's medium caused a marked increase in the virulence of strains of *Clostridium chauvei* inoculated on such media.

23. Generalized Torula Infection in Man. B. Z. Rappaport and Bertha Kaplan, University of Illinois, College of Medicine.

A study of the thirteenth case of torula infection in man reported in the literature. Clinically a chronic meningitis, diagnosed antemortem by positive blood and spinal fluid cultures. Report of the pathological findings.

24. Influence of B. Welchii Toxin on Erythrocytes in Vivo and in Vitro. Guilford B. Reed and J. H. Orr, Queen's University, Kingston, Canada.—(Lantern.)

B. Welchii toxin injected intravenously into rabbits produces rapid destruction of red-blood cells. If the dose of toxin is large the destruction is rapid and may be followed by the death of the animal. If the dose is smaller the destruction is less rapid and the anemia may be transient. In either case, however, the red-cell destruction is accompanied by a profound alteration in the structure of the cells characterized in the first stages of the toxemia by an increase in microcytes followed later by an increase in macrocytes, poikilocytes, polychromatophilia and budding-cells.

Similar changes are observed when *B. Welchii* toxin is mixed with defibrinated blood or with washed erythrocytes suspended in physiological salt solutions.

25. Pernicious Anaemia-like Blood Changes Accompanying B. Welchii Infections in Rabbits. Guilford B. Reed and J. H. Orr, Queen's University Kingston, Canada.—(Lantern.)

A virulent *B. Welchii* culture kept for several days in the ice box and injected in suitable numbers into rabbits subcutaneously, intramuscularly or intravenously produces an infection which may terminate fatally in from twelve hours to three weeks or may persist for two to five weeks and may be followed by apparently complete recovery of the animal. Such infections in rabbits are always followed by an anaemia the extent of which is roughly proportional to the activity of the infection.

Where the *B. Welchii* infection is very active there is a marked decrease in the number of circulating erythrocytes, and increase in the color index and a profound alteration in the structure of the red-cells characterized by anisocytosis, poikilocytosis, polychromatophilia and nucleated red-cells. Where the infection is less active there may be no decrease in red-cell count but at the same time a measurable anisocytosis and poikilocytosis.

26. Hemolytic Streptococci of the Beta Type in Certified Milk. J. HOWARD BROWN, W. D. FROST AND MYRTLE SHAW, University of Wisconsin.

Over 90 strains of beta hemolytic streptococci were isolated from the cows and mixed milk of a number of certified milk herds including about 900 cows. There was nothing abnormal about the condition of these dairies and the milk is not known to have caused any infection in the consumers. The streptococci were carefully studied in comparison with strains of *Streptococcus epidemicus* from most of the well studied milk-borne epidemics of septic sore throat which have occurred in the United States since 1911. All the strains of *S. epidemicus* were found to be alike in being encapsulated, in producing rapid hemolysis of red blood cells in fluid media, in being pathogenic for mice, in producing a final hydrogen ion concentration not higher than pH 4.8 in glucose broth, in fermenting salicin but not mannite, and in failing to hydrolyze sodium hippurate. All of the bovine strains differed from *S. epidemicus* in one or more of the above characters. There is no evidence that any of them are pathogenic for man. No known single test is sufficient to differentiate the bovine strains from those which may be pathogenic for man.

Hemolytic streptococci, non-pathogenic for man, are to be found in the milk of many clinically normal cows and probably in the mixed milk of all herds of more than a few cows.

The streptococcus of milk-borne septic sore throat is a well defined species or variety which may be distinguished from other hemolytic streptococci found in milk by the tests described. It retains its characters unchanged during years of cultivation in the laboratory.

27. Studies on the Streptococcus of Epidemic Encephalitis. ALICE C. EVANS AND WALTER FREEMAN, Hygienic Laboratory, Washington, D. C.

A streptococcus was obtained from the nasal washings during life and from the midbrain and heart's blood taken at necropsy from a human case of epidemic encephalitis. This streptococcus is highly virulent for rabbits when inoculated into the brain, causing death in from five and three-quarters to 18 hours when the inoculating dose is 0.25 cc. of undiluted broth culture. When inoculations were intracerebral the minimal lethal dose was found to be 0.25 cc. of culture diluted 1 to 10,000. Occasionally infection followed heavy intravenous

inoculations. In that case the brain was always found to be heavily seeded with the streptococcus.

Filtrates of an emulsion of the human brain, of the emulsions of rabbits' brains, and filtrates of cultures have given uniformly negative results when inoculated directly into the brains of rabbits. By planting the filtrates in meat medium, however, virulent cultures have been obtained repeatedly, and they have shown that the organism is in a filterable form in the human brain, in the rabbit brain, and in cultures.

The experimental disease produced in rabbits and monkeys closely resembled epidemic encephalitis of man. The symptoms and histological lesions are discussed.

The same streptococcus was also obtained from the nasal washings, of saliva, or both, from all of the three other patients examined, who were suffering with the chronic form of the disease and from the throats of normal persons.

Serologic tests have not yet been made.

The streptococcus here described was identified with the organism obtained from a human case and described by von Wiesner as the cause of epidemic encephalitis. It agrees also with the streptococcus which Rosenow has obtained repeatedly from human cases. Culturally there is no characteristic which would distinguish it from an ordinary *Streptococcus viridans* but morphologically it exhibits a variety of characteristic pleomorphic forms.

28. Further Studies on the Poliomyelitis Precipitin Reaction. E. C. Rosenow, Department of Experimental Bacteriology, Mayo Foundation, Rochester, Minnesota.

In this study previous findings are corroborated.

The incidence of a positive precipitin reaction at the time of attack of poliomyelitis was almost 100 per cent whereas the reaction almost always became negative within three weeks after onset of attack. The results in persons having abortive attacks were similar. The incidence of positive reactions in normal contacts was 77 per cent; in normal controls within the epidemic zone, 72 per cent; outside of epidemic zone, 18 per cent; and in isolated family groups at the time of epidemics, was on the average about ten times as high as after the epidemic had disappeared. A positive precipitin test with swabbings from the throat indicates the presence of the characteristic streptococcus as determined by agglutination reactions and the production of flaccid paralysis in animals.

29. The Precipitin Reaction in the Diagnosis of Scarlet Fever and Allied Hemolytic Streptococcus Infections. E. C. ROSENOW, Department of Experimental Bacteriology, Mayo Foundation, Rochester, Minnesota.

The precipitin reaction with an immune serum, prepared by the intravenous injection of first, the dead bacteria and the living cultures of the hemolytic streptococcus from scarlet fever, has been found valuable in differentiating ordinary hemolytic streptococcus infection of the throat and infections due to scarlatinal hemolytic streptococci. A close agreement was found between the results of the precipitin reaction and the production of toxin neutralizable by scarlatinal immune serums. Infection of the throat due to scarlatinal hemolytic streptococci was found common at the time of an epidemic in persons who had had attacks of scarlet fever and others in whom the Dick skin test was negative. A close relationship has also been found between virulence and toxin producing power, and scarlatinal and other hemolytic streptococci. Some of the former lost toxin producing power and some of the latter acquired the power to produce toxin neutralizable by scarlatinal antistreptococcus serum.

30. Toxin Production of the Streptococcus erysipelatis. KONRAD E. BIRKHAUG, Department of Bacteriology, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.

The toxins employed in these studies were prepared in Douglas' tryptic medium inoculated with cultures of *Streptococcus erysipelatis*, which were isolated from the erysipelatous lesions of patients ill with erysipelas. Among thirty-four strains grown at 37°C. for periods varying from six to ninety-six hours, the maximum toxin production was obtained in lots incubated for about 48 hours. Each of the 34 strains studied was found to yield uniformly toxic filtrates. A skin test dose of 0.1 cc. of a 1:1000 dilution of erysipelas toxic filtrate produced in the skin of susceptible persons a lesion, similar in nature to that obtained in the Schick and Dick tests, which measured more than 1.5 cm. in diameter. Complete neutralization of one skin test dose of the erysilepas toxin was obtained by mixing it with an equal amount of convalescent erysipelas serum, or with 0.001 cc. of erysipelas antistreptococcic rabbit or donkey sera. Neutralization of the erysilepas streptococcic toxin was not accomplished by Dochez' scarlatinal antistreptococcic serum, nor by normal rabbit or donkey sera. During the acute stages of erysipelas the patient's blood serum and urine contained a toxic substance which was

completely neutralized by convalescent erysipelas serum and which disappeared from the patient's blood serum and urine as soon as twelve hours after the administration intramuscularly of 25 to 100 cc. of erysipelas antistreptococcic rabbit or donkey sera. If the disease persisted unchecked by the serum therapy, the skin reaction remained positive until defervescence and definite regression of the erysipelatous lesion occurred.

Positive skin reactions were obtained by one skin test dose of erysipelas streptococcic toxin in 27 per cent of apparently normal adults and in 21 per cent of normal school children. Among 19 persons with definite histories of single and recurrent attacks of erysipelas, 4 persons gave positive reactions with one skin test dose of the erysipelas streptococcic toxin. These findings add further evidence to our previous reports that a specific relationship exists between *Streptococcus erysipelatis* and erysipelas.

31. Studies on Scarlet Fever Toxin. PAUL F. ORR, AND M. S. MARSHALL, State Department of Health, Lansing, Mich.

Scarlet fever toxin was produced in accordance with the procedures of the Doctors Dick from a selected group of strains of hemolytic streptococci isolated either from the throats of scarlet fever patients, or from postscarlatinal discharging ears.

Twenty or thirty minutes exposure of the toxin at 100°C. usually rendered it atoxic; however, at least 45 minutes is necessary to insure 100 per cent negative controls in the skin test. Culture medium, treated as in the preparation of toxin, except for the inoculum, gave no reaction in any skin tests in which it was used as a control. It is not considered as good a control as heated toxin.

The age grouping of individuals susceptible to scarlet fever as evidenced by a positive Dick test showed a maximum susceptibility at from two to four years of age. However, the number of tests on children under three years was relatively small, and it is possible that the maximum susceptibility may occur in a younger age group. An average of 34 per cent of positive Dick reactions was obtained in individuals of all age groups in State institutions.

Very little difficulty was experienced in reading skin tests on colored children.

Some work has been done by the Department on the dosage of toxin necessary for immunization, demonstrating the necessity for a large number of skin test doses for injection subsequent to the first.

32. Immunization Against Diphtheria and Scarlet Fever With Toxins Detoxified With Sodium Ricinoleate. W. P. LARSON, Department of Bacteriology and Immunology, University of Minnesota.

Mechanism of detoxification. Quantitative relationship between toxin and soap. Effect of concentration of both toxin and soap. The effect of dilution upon a neutral soap toxin mixture. Results of animal experimentation. Results of active immunization against diphtheria and scarlet fever. The possibility of suppressing epidemics of scarlet fever by active immunization.

33. The Bacteriology of Chronic Prostatitis and the Localization in Animals of Organisms Isolated. A. C. NICKEL, Department of Experimental Bacteriology, Mayo Foundation, Rochester, Minnesota.

This paper takes up first, the bacteriology of chronic prostatitis as reported by various investigators, and then compares those bacteriological findings with the results obtained by the author. A further study is made of the relation of chronic prostatitis to systemic conditions and to other foci of infection, and the causal relation of the bacteria to these conditions as found by experimentation, is based upon the elective localizing power of the microbe in inoculated animals.

34. The Distribution of Fusiform Bacilli and Spirochetes in Tissue. I. PILOT, University of Illinois, College of Medicine.

In pathological lesions the fusiform bacilli and spirochetes have often a characteristic distribution. The bacilli lie in the necrotic portion while the spirochetes are seen advancing from the margins into the surrounding tissues.

A STUDY OF BACILLUS ACIDOPHILUS FROM THE DIGESTIVE TRACT OF CALVES

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In investigations (Smith and Orcutt, 1925) carried on upon the early diseases of young calves, notably an early diarrhea known as scours the regular presence of certain Gram-positive bacilli and cocci in the normal digestive tract made it appear desirable to study these forms in detail. Two forms were manifestly predominant according to films from various levels of the digestive tract although other species, notably sporebearing anaerobes, were present in small numbers. The present article deals with the dominant Gram-positive bacillus, *Bacillus acidophilus;* the following paper with a second common form, designated as the enterococcus.

The literature contains only a few references to acidophilus bacilli from calves. Küthe (1915) made a study of bacteria in the intestinal tract using material from the stomach, duodenum jejunum, ileum, cecum, colon, and rectum. He noted the appearance and reaction of the contents, made direct microscopic examination of stained smears, and cultured the material aerobically and anaerobically using plain agar plates, acetic acid bouillon, and deep layers of sugar agar. He always found a bacillus which he called *Bacillus acidophilus-polymorphus.* He noted the bacilli in the smears from the contents, usually of all levels, and he cultivated them in good numbers in the acid bouillon while the development in the non-acid cultures was more irregular and uncertain.

The present work was done with material from segments of the small intestine from the duodenum to the ileocecal valves and

usually with material from the fourth stomach and sometimes from the cecum. The calves represent two groups, namely, 8 normal calves, from one to two days up to two to three months of age; and 14 calves having diarrhea, from one to eight days old. These calves had received a milk diet. Among all animals of both groups Gram-positive rods appeared in varying numbers throughout the segments. Usually they were very numerous in the fourth stomach and the predominating type in that region. In the normal cases they were the predominating type throughout the small intestine, or they occurred in more or less equal numbers with Gram-positive cocci. In cases with scours a colon type of organism predominated in the lower segments (Smith and Orcutt, 1925).

In the beginning of the work the material was cultured on standard agar plus horse blood and only a few acidophilus colonies developed on this medium. The reason for the small numbers was probably due to the use of a too alkaline medium. The necessity of a certain degree of acidity for the cultivation of these forms was shown by the results obtained by varying the reaction of the medium, in 2 cases in which according to the films *Bacillus acidophilus* was present in large numbers.

These results indicated that the *B. acidophilus* types were living throughout the small intestine and that ordinary standard agar adjusted to a suitable acidity (pH 6.8) plus horse blood furnished a good medium for the isolation of this organism. Although pH 6.8 has proved a favorable H-ion concentration for an abundant growth, if the acidophilus bacilli were mixed with colon types the agar could be adjusted to pH 5.0, which is still favorable to *B. acidophilus* but harmful to *B. coli*.

The question arose as to whether the acidophilus bacilli actually multiplied in the intestine or were only carried down mechanically from the fourth stomach. To answer this three normal calves were examined carefully by plating measured amounts of material from the fourth stomach and the segments of the small intestine and counting the colonies which developed. All cases gave similar results, two of which are tabulated.

The figures show that in the segments just below the stomach

B. acidophilus occurs in smaller numbers than in the fourth stomach. However, they also show that the acidophilus bacilli are present in large numbers throughout the small intestine and that in every case they exist in greater numbers in the lower segments than in the duodenum. These results suggest that *B. acidophilus* multiplies in the small intestine. The amount of increase varied in the different segments of the cases observed,

TABLE 1*

CALF	REGION	OCCURRENCE (IN FILMS)	CULTIVATION (STANDARD AGAR PLUS BLOOD)
			pH 7.4–7.8
1065	Fourth stomach	++++	++++
	Duodenum	+++	+
	Second segment	+++	0
	Third segment	++	0
	Fourth segment	+++	+
	Fifth segment	+++	0
	Sixth segment	+++	0
	Ileum	++++	+
	Cecum	++++	+
			pH 6.8
1106	Fourth stomach	++++	++++
	Duodenum	+++	+++
	Second segment	+++	+++
	Third segment	+++	++++
	Fourth segment	+++	+++
	Ileum	++	+++

* ++++ indicates large numbers; +++, fair numbers; ++, moderate numbers; +, a few; 0, not cultured.

probably depending on conditions existing in the different regions at different times. Also the multiplication in the intestine is not as abundant as in the fourth stomach although a certain amount does occur.

This group of organisms was first isolated and named by Moro (1900). All the earlier reports describe the strains as producing acid but no gas from carbohydrates. Torrey and Rahe (1915) described a gas-producing type which they named *B. acidophil-aerogenes*. They isolated this gas-producing type from the feces of man, sheep, and hens. They described two types of colonies

appearing on glucose oleate agar plates, and also noted that in sugar bouillon the gas types were inclined to grow in particles adhering to the sides and bottom of the tube and tended to produce more acid than the non-gas types. The amount of gas produced varied with different strains and also from time to time with the same strain. The maximum gas production reported was 60 per cent and the minimum 2 per cent. They stated that the gas was chiefly hydrogen and that the ratio of H to CO_2 was 4:1 or 6:1. Later Rahe (1918) presented a classification of aciduric bacteria. He named four groups: *B. bulgaricus*, *B. bifidus*, *B. acidophilus*, and *B. acidophilaerogenes*. These were

TABLE 2

CALF	REGION	NUMBER OF B. ACIDOPHILUS PER CUBIC CENTIMETER
1106	Fourth stomach	1,330,000
	Duodenum	261,000
	Second segment	859,000
	Third segment	4,000,000
	Ileum	700,000
1112	Fourth stomach	7,820,000
	Duodenum	930,000
	Second segment	1,642,000
	Third segment	1,865,000
	Fourth segment	5,224,000
	Ileum	690,000

subdivided into classes according to the fermentation of various sugars. The acidophil-aerogenes type was the gas-producing group and *B. bulgaricus* was separated from the others by its failure to ferment maltose.

Recently Cannon (1924) reported a biologic study of aciduric bacteria. He referred to the three main groups described in the literature, namely, *B. acidophilus* (Moro), *B. bifidus* (Tissier) and *B. acidophil-aerogenes* (Torrey and Rahe). Cannon worked with 64 strains of *B. acidophilus* and 34 strains of *B. acidophil-aerogenes* isolated chiefly from sputum and feces of normal human adults. None of his strains were obtained from calves. He described the bacilli as facultative anaerobes but growing well

under aerobic conditions. They did not liquefy gelatin. Some strains coagulated milk and some did not. In his study on the effect of the hydrogen-ion concentration he reported that a pH between 6.0 and 7.0 was most favorable but the majority of cultures grew on media adjusted from pH 4.8 to 7.6.

Küthe's paper (1915) is the only report describing acidophilus types from calves. The chief form which he found he called *B. acidophilus-polymorphus*. This organism he described as a Gram-positive rod with rounded ends often occurring in long chains and sometimes in single cells. These shorter single forms were sometimes slightly motile. On agar it formed dewdrop-like colonies which, when magnified, appeared like anthrax colonies. It also grew in a deep layer of glucose agar appearing in twenty-four hours as small dots, and when magnified the colonies showed smooth or slightly notched edges. In a stained preparation from an anaerobic colony the bacilli were thick and occurred singly and in pairs; sometimes there was a long thick rod with a short form above it like a head, also some comma forms and swollen rods. When transplanted back again under aerobic conditions the colonies were of the grayish dewdrop appearance and the bacilli grew again in chains, but the elements were thicker and more irregular than in the first aerobic culture. The alternate cultivation from anaerobic to aerobic conditions could be continued through a long series. Küthe considered *B. acidophilus-polymorphus* to be a single organism characterized by great pleomorphism and variation from aerobic to anaerobic growth. He further described this bacillus as coagulating milk and reddening litmus whey in twenty-four hours. It did not grow on gelatin and its development on serum was doubtful. It produced no indol and formed no gas. Küthe also mentioned two other rarer acidophilus types. These developed anaerobically and one type failed to grow in aerobic cultures.

The present report on calf acidophilus types includes studies of 17 strains recently isolated and 3 strains which were isolated from calves in 1917–1919 by Marian S. Taylor and have been kept in the collection of stock cultures here since that time. The three earlier strains and 12 of the recent strains belong to the class

designated by Rahe, and later referred to by Cannon as *B. acidophilus;* and 5 strains, because of the production of gas, fall in the group called *B. acidophil-aerogenes.* In the *B. acidophilus* group 5 strains were isolated from the 4th stomach, one from the 4th segment, and one from the ileum, all from different calves; and 5 strains came from the duodenum of three different calves. In the *B. acidophilaerogenes* group 2 strains came from the 4th stomach and 3 from the ileum.

All strains have certain morphological characters in common. They are all strongly Gram-positive rods, rather broad, and with blunt or square ends, showing considerable variation in length of elements. Some rods are short, some long, and some occur in still longer filaments. The rods are single, paired, or in short or long chains, sometimes twisted and tangled. Certain differences occur among the strains. Some always appear as rather short forms usually in pairs, short chains, and groups, and often in parallel arrangement but with a certain variation in the length of the rods. Other strains show many long forms and develop long chains. These rods are usually straight and broad but the longer chains are often twisted or tangled. The gas-producing strains show the greatest variation. They are likely to show more curved rods and the chains are more tangled.

The growth of these strains has been observed in various media and the fermentation reactions of certain carbohydrates recorded In the first place on the blood agar plates, which were used for the isolation of the cultures, all strains, both gas and non-gas types, showed a similar appearance. In twenty-four hours very tiny colonies with tiny greenish zones developed. Some colonies were only visible with the hand lens. In forty-eight hours the colonies were still small and showed small zones of greenish discoloration. The deep colonies often appeared irregular or jagged. The form of the surface colonies was seen better on the plain agar plates. From the different strains two or possibly three types of surface colony could be distinguished. All colonies were small delicate growths, usually not over 1 mm. in diameter. A few strains developed round, even, rather opaque colonies; other strains formed thin, delicate, irregular colonies; and the

remaining strains developed small, delicate, more or less round colonies, but when examined with a hand lens threads were seen projecting from the edges. The round and irregular colonies occur in both gas and non-gas producing types. In the same strain some colonies may be more jagged than others.

In standard bouillon (pH 6.8) the strains usually produce a small amount of fine sediment and a clouding made up of very fine suspended particles. In sugar bouillon the clouding is usually heavier and particles sometimes adhere to the sides of the tube. This is always the case with the gas strains. Certain strains do not cloud the bouillon but grow as a soft sediment made up of small particles. The strains which produced the sediment in bouillon also developed round colonies, but all strains forming round colonies did not grow as a sediment in bouillon.

All these acidophilus strains were grown in tubes of glucose agar 12 cm. deep. The cultures were inoculated when the agar was still soft after boiling. The medium was quickly solidified without shaking and incubated. The growth occurred throughout the depth of the agar. The colonies developed equally well from the top to the bottom of the tube, suggesting capacity for anaerobic growth.

An experiment with milk cultures was made by inoculating a series of milk tubes with different amounts of a bouillon culture and determining the pH values and the time when coagulation appeared. The amounts of culture used for inoculation were 0.01, 0.05, 0.1, 0.2, and 0.3 cc. The results showed that the tube inoculated with the largest amount of culture was the first and the one inoculated with the smallest amount the last to coagulate. The first sign of coagulation occurred in twenty-four hours in the tube receiving 0.3 cc. No coagulation was visible in the tube inoculated with 0.01 cc. until the 11th day. The coagulation always appeared first at the bottom of the tube. The original pH value of the milk was 6.7, and the H-ion concentration gradually increased. The greater the original inoculation the more rapidly the acidity increased. No visible coagulation occurred until a pH of about 5.8 was reached. A firm coagulum throughout the tube occurred at about pH 5.5 and the

acidity might continue to increase gradually up to pH 4.8. The rate of coagulation depended upon the amount of culture inoculated. The greater the number of bacilli added the more rapid the coagulation; but regardless of the amount of the inoculation, no coagulation was visible until a rather definite pH value was reached, and the coagulation was not complete until a second definite H-ion concentration was attained. In other words, the time factor of coagulation varied with the amount of culture transferred, but the relation between the pH values and the degree of curdling was constant, the curdling always appearing at a rather definite H-ion concentration. On the other hand, the final pH value attained was also variable according to the amount of material inoculated. All tubes inoculated with amounts of 0.05 cc. and below reached a final pH of 5.4 to 5.3 after three weeks, whereas the tubes inoculated with 0.1 cc. or more reached within the same period a final pH of 4.8. A similar test was carried out with additional strains to see if this difference in final H-ion concentration was general. Seven strains were used and the amounts inoculated were 0.2 and 0.02 cc. of a bouillon culture. In every case the same conditions occurred as in the first experiment; namely, no visible coagulation until the pH value was about 5.8; the coagulation was not complete until the pH value was about 5.5; and the final H-ion concentration after three weeks gave a pH of 5.3 to 5.1 for the tubes inoculated with 0.02 cc., and a pH of 4.8 to 4.6 for the tubes inoculated with 0.2 cc.

All the cultures grew in gelatin at 37°C., and after a month no liquefaction occurred. The gelatin solidified as soon as it cooled.

Some experiments were made to determine the effect of the hydrogen-ion concentration on the growth of these cultures. The limiting H-ion concentrations were determined and also the range giving the best growth. The procedure used was to prepare a series of tubes of bouillon with a pH range from 8.0 to 4.4. Standard bouillon was used and N/20 NaOH added to obtain pH values above 7.6, and acetic acid added for the pH values below 7.6. Each tube contained the same amount of medium and was inoculated with the same amount of a forty-eight hour agar growth suspended in sterile distilled water. The growth

was measured by plating and counting the colonies and observing the clouding of the bouillon. The medium used for plating was standard agar pH 6.8 plus horse blood. The colonies were counted after 72 hours incubation. This experiment was made with five strains: two gas-producing strains, 1071 and 1106 (fourth stomach); and three non-gas types, 1078, 1106 (ileum), and 133. Strain 1078 represented the cultures forming more or less even colonies and producing a clouding in bouillon; strain 1106 (ileum) represented the cultures forming round colonies and growing as a sediment in bouillon; and, finally, strain 133 was used as a representative of those which had been under cultivation since 1917. The results for the different pH values, indicated by the appearance of the bouillon and the colony counts, showed that *B. acidophilus* develops over a fairly wide range of H-ion concentrations. The recently isolated strains, including the gas and non-gas types, gave a similar range, but the older strain differed somewhat. In every case there was no growth at pH 8.0 and 4.4. Among the recently isolated strains there was little or no growth at pH 7.7 to 7.8, which indicated that the limiting H-ion concentration on the alkaline side was pH 7.8 to 8.0. Also among the recently isolated strains there was always some growth at pH 4.6, and the gas strains showed a better development than the non-gas types at this H-ion concentration. There was usually a good growth at pH 4.8, but the maximum development occurred between pH 7.0 or 6.8 and pH 5.2. Strain 133 agreed with the other strains in its limiting H-ion concentration on the alkaline side but it had a higher pH value as the acid limit. This strain had been kept on standard agar of about pH 7.4, and this may account for the fact that it was less resistant to acid than the recently isolated strains. Its limiting H-ion concentration on the acid side was pH 5.2 to 5.0. The range for good growth was between pH 7.7 and 5.5. The optimum growth occurred from pH 7.4 to 6.0.

The fermentation reactions of these acidophilus strains were tested on glucose, lactose, sucrose, maltose, and mannitol. Fermented bouillon (pH 6.8) plus 1 per cent of the sugar was used for these tests. One lot of bouillon was used throughout for each

set of sugars. The pH values were obtained by the micro-colorimetric method described by Brown (1924). The per cent of acid produced was also obtained by the usual titration method. All strains produced a certain amount of acid from glucose, lactose, sucrose, and maltose, usually the greatest amount from glucose and the smallest amount from maltose. The gas-producing strains formed as a rule slightly more acid in glucose, lactose, and sucrose, and considerably more in maltose than the non-gas producing types. Only one strain formed a small amount of acid from mannitol. Ten strains were tested in duplicate with 1 and 2 per cent of glucose. After two weeks the results in 7 cases showed practically the same pH values for the two tubes, and in the other 3 cases a slightly greater amount of acid occurred in the tubes containing the 2 per cent of sugar, the difference being the greatest in the gas-producing strains.

In regard to the relative amount of acidity produced the individual strains showed some differences. The titration results were rather more variable than the pH values. Kendall and Haner (1924) in a recent study of the metabolism of *B. acidophilus* refer to three morphologically different types. Two of their types fermented the same sugars and the third type fermented two additional ones, sorbitol and mannitol. In regard to the relative amounts of acidity formed by each type in milk, and in gelatin containing glucose, lactose, and sucrose, they noted certain differences in the quantity of titratable acid produced and also in the rate of acid production. They mentioned that a resultant between the two factors, inherent acidogenesis and cultural adaptability, was the probable explanation of these differences.

Another reaction, which differs from time to time and varies for different cultures of the same strain, is gas production. The acidophilaerogenes strains are quite variable in the amount of gas produced and in the time when gas appears. Thirty or more different records of strain 1071 in fermented bouillon plus 1 per cent glucose in fermentation tubes have been made. Just before these inoculations the strain was plated and a single colony picked in a series of five successive transfers. Each time the

plates appeared pure. An agar slant was inoculated from a colony on the fifth plate and a transfer from this culture was used to inoculate the fermentation tubes. The following examples are mentioned to show the extent of the variations occurring in one strain. Two tubes of approximately the same size and filled to about the same level with one lot of bouillon were inoculated with equal amounts of material from the same culture. In one tube 4 per cent gas was formed in forty-eight hours and a total of 37 per cent appeared in six days; while in the second tube there was only a bubble of gas on the eleventh day and no further increase after fifteen days. A third tube was inoculated from the second and showed no gas after twelve days, although growth occurred as usual; and a fourth tube, inoculated from the third, again contained a considerable amount of gas. In another series, cultures were inoculated from the bulb, neck, and arm of a fermentation tube culture which contained gas. The results showed one culture inoculated from the neck which failed to produce gas, and a second tube inoculated from the same region which contained 34 per cent gas. All the tubes inoculated from the bulb and arm contained gas but in amounts varying from 10 to 31 per cent. Thus even with uniform conditions of the medium and the material for inoculation, the rate and amount of gas production still varied in different tubes. Also cultures containing little or no gas may give rise to cultures producing considerable gas and, *vice versa*, cultures containing gas may give rise to others with little or no gas. Similar variations occurred in two other gas-producing strains tested in shorter series. Torrey and Rahe (1915) also reported variability in the amount of gas produced. However, the calf strains differ from the organisms studied by these workers in the composition of the gas. Torrey and Rahe state that the gas was chiefly hydrogen, but do not mention their method of measuring the gas. In the calf strains the gas, as it is formed in the fermentation tube, is almost totally absorbed by NaOH solution, indicating that it is chiefly CO_2.

An experiment was made by growing *B. acidophilus* and *B. coli* together in mixed culture. Fermented bouillon (pH 7.4) plus 1 per cent glucose was inoculated with a known number of

B. coli and *B. acidophilus*. Pure cultures of each organism were made as controls. The growth and pH values were watched and it was found that *B. coli* grew more rapidly in the beginning, but when the pH value reached 5.2 to 5.0, which occurred after the first twenty-four hours, then *B. coli* ceased to multiply and *B. acidophilus* continued to increase. On the third day the control tube of *B. coli* alone showed the bacilli present in larger numbers than in the mixed culture. The counts gave 2228 M. *B. coli* per cc. in the control, in contrast to 32 M. per cc. in the mixed culture, while *B. acidophilus* was apparently still increasing in the latter. Thus *B. acidophilus* was able to outlive *B. coli* in the acid condition. On the other hand, if no fermentable substance is present, as indicated in a similar experiment of mixed cultures in plain bouillon, then *B. coli* will keep the pH value 7.0 to 7.5. This H-ion concentration is unfavorable for *B. acidophilus* but suitable for the growth of *B. coli*. In this experiment *B. coli* multiplied greatly and continued to live in large numbers for a week, keeping the ascendency over *B. acidophilus*. These results indicate that in mixed cultures *B. acidophilus* is able to check the growth of colon bacilli only when a fermentable substance is present. They are in agreement with Cannon's experiments with mixed cultures of *B. coli* and *B. acidophilus*. Similar experiments with mixed cultures of enterococci and colon bacilli, and also with mixed cultures of the three types, *B. acidophilus*, enterococci, and *B. coli*, are reported in a paper on enterococci from calves.

CONCLUSIONS

1. *B. acidophilus* types are always present throughout the small intestine of young calves and are the predominating forms in the fourth stomach. In normal animals they are the predominating organism in the small intestine or they occur in more or less equal numbers with Gram-positive cocci.

2. *B. acidophilus* multiplies in the small intestine of normal calves but the degree of multiplication is greater in the fourth stomach than in the intestine.

3. Ordinary standard agar of pH 6.8 to 5.0 plus horse blood

proved a suitable medium for the isolation of these organisms. Agar pH 6.8 is the most favorable for abundant growth but the more acid agar is better whenever colon organisms are numerous.

4. All the recently isolated calf strains showed certain characters in common, such as (*a*) similar optimum and limiting H-ion concentrations; (*b*) development of small greenish zones on blood agar; (*c*) growth throughout a layer of agar 12 cm. deep; (*d*) no liquefaction of gelatin; (*e*) coagulation of milk at definite pH values and final H-ion concentrations after three weeks, varying with the amount of material inoculated; (*f*) production of acid from glucose, lactose, sucrose, and maltose. The strains differed in the fermentation of mannitol; one strain produced a small amount of acid from this sugar and all the other strains failed to ferment it.

5. The calf strains may be divided into two groups according to gas production: (*a*) non-gas types, *B. acidophilus;* and (*b*) gas types, *B. acidophilaerogenes.*

6. *Acidophilaerogenes* strains are characterized by variation in the rate and amount of gas production, and the gas is always CO_2.

7. Experiments with *B. acidophilus* and *B. coli* in mixed culture, taken together with certain biological characters, indicate that *B. acidophilus* possesses a greater resistance to acid than *B. coli.*

8. The calf strains correspond in general in biological characters to *B. acidophilus* types isolated from man and other animals by earlier observers.

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A STUDY OF ENTEROCOCCI FROM THE DIGESTIVE TRACT OF CALVES

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A group of organisms described as Gram-positive cocci usually occurring in pairs and commonly found in the intestinal flora has been referred to under various names. Schmitz (1912) mentioned that the German literature contained references to *Micrococcus ovalis* (Escherich) and *Streptococcus lacticus* (Kruse) but seldom referred to enterococci. Thiercelin named this type of organism the enterococcus and hence in French publications this name is generally used.

Only brief references to the enterococcus from calves have been found. Küthe (1915) in his study of the bacteria of the intestinal tract of young calves states that cocci of various forms were frequently present in the intestines. These cocci were Gram-positive and grew well in acid bouillon. Tricoire (1917) in his review of the enterococcus states that enterococci occur in the intestines of cattle and some other animals. He described the organism as a micrococcus intermediate between streptococci and pneumococci, and appearing in animals and young cultures as a diplococcus, sometimes with a capsule or halo. The two cocci might be alike or different in size and form, and joined either in a straight line or at an angle. In older cultures the cocci were grouped in masses or chains and in very old cultures rod-like forms were found.

In the present study the same animals and material were used as described in the paper on *B. acidophilus* in calves (Orcutt, 1926); namely, the 4th stomach and small intestines, and sometimes the cecum, from a group of normal calves of 1 to 2 days up

to 2 to 3 months of age, and similar material from sick calves 1 to 8 days old. Preparations from the intestinal contents stained by the Gram method showed Gram-positive cocci, usually in pairs. The cocci were sometimes slightly elongated and the two elements of a pair sometimes varied in size and shape. A small lighter area might exist around the cocci with a line between the two elements of a pair, suggesting a capsule, but this was not always the case. Also the two cocci in the pair were arranged usually in a straight line but sometimes they were at an angle.

These cocci were present in considerable numbers in the films from practically all intestinal levels of normal calves. They also existed in varying numbers in the intestinal contents of calves suffering from diarrhea. In some cases rather large numbers, and in other cases only a few cocci were seen. The distribution of the cocci as indicated from the films showed that as a rule they were more abundant in the small intestine than in the 4th stomach although a few cases showed the cocci about equally numerous in both regions.

The development of the cocci on plates inoculated from the intestinal contents was variable and sometimes the growth did not correspond to the numbers seen in the films. Also, occasionally from a certain segment the cocci developed in almost pure culture. This indicated that multiplication could occur in the intestine, and the region where abundant growth took place probably depended upon the presence of substances which were favorable to the growth of the cocci.

These Gram-positive diplococci seen in the intestinal contents of the calves have been considered as enterococci. They form one of the groups of organisms always present in the intestinal tract of young calves and their existence there is probably a normal condition. They seem to be only saprophytic organisms, but according to a review by Tricoire they are able at times to produce disease. Tricoire (1917) stated that the enterococcus was a saprophyte of man and animals but that it might become pathogenic. He mentioned that it existed normally in the intestinal tract and was one of the fundamental elements in the intestinal flora together with *B. coli* and *B. bifidus*. He listed various

reported cases of enterococcus infection, including gastro-enteritis, hepatic infections, appendicitis, peritonitis, intestinal intoxications, lung abscess, rheumatism, and also cases of tuberculosis, typhoid and paratyphoid fever with which the enterococcus was associated. Furthermore the organism has been reported in wound infections. Thus numerous cases are on record in which infection is ascribed to an organism designated as enterococcus. In the intestines of the calves used in this study the enterococci occurred in considerable numbers, but there was no disturbance referred to their presence.

Tricoire (1917) described the enterococcus as a facultative aerobe growing at room temperature but with an optimum temperature for growth of 37°C. It developed readily in bouillon and pepton solution, and after 24 to 48 hours it produced a sediment, rising in a spiral without breaking up when shaken. It coagulated milk, did not liquefy gelatin, formed no indol, and generally did not ferment sugars.

Dible (1921) considered the enterococcus of Thiercelin, the *Micrococcus ovalis* of Escherich, and the *Streptococcus fecalis* of Andrewes and Horder as probably belonging to the same group of organisms. He studied a large number of strains isolated from feces and concluded that the enterococci were a rather well defined group which could be differentiated from the streptococci by resistance to heat. He designated a central type and three variants. He described the enterococcus as a lanceolate diplococcus growing in bouillon, sometimes in short chains. Occasionally a slight halo was seen around the cocci, but no capsule was demonstrated. It grew slightly in media alkaline to phenolphthalein and gave no growth in media acid to Congo red. In bouillon it formed a uniform turbidity. It coagulated milk and did not liquefy gelatin. It was insoluble in bile and had no action on red blood corpuscles, producing neither hemolysis nor methemoglobin. The main type fermented lactose, salicin, sucrose, and mannitol. One variant failed to ferment sucrose, a second failed to ferment mannitol, and a third failed to ferment both sucrose and mannitol. None fermented raffinose and inulin. Besides these four groups he also mentioned an occasional variant

capable of fermenting raffinose. He stated that enterococci occurred constantly in stools but were not particularly associated with diarrhea. In a few instances he found the organism in saliva.

Kendall and Haner (1924) studied the metabolism of *Micrococcus ovalis*. They state that Escherich isolated and described this organism in 1886, and that in 1899 Thiercelin rediscovered it and named it enterococcus. Kendall and Haner isolated enterococci frequently from the duodenum of adults. Their cultures represented the four types of Dible and also three additional variants. They considered the fermentation of salicin of importance for identification. They found certain strains which fermented inulin but differed from pneumococci by being insoluble in bile. They considered that the enterococci were normal intestinal microbes of the lactic acid forming type.

The present study comprises twelve cultures from calves. They showed certain differences among themselves. One form agreed with the type organism of Dible. There were three variants, one like Dible's variant I, a second like Kendall and Haner's variant IV, and the third and largest group like variant V of Kendall and Haner.

Their appearance in films from intestinal contents has already been mentioned. In 24 or 48-hour agar cultures all strains gave a similar picture. The cocci were mostly in pairs, scattered or grouped together, and some short chains were also seen. The elements were round or slightly elongated and rather irregular, frequently varying in size. An examination of preparations made with India ink showed no distinct capsules. In plain bouillon the individual strains showed certain differences. In some cultures the cocci were chiefly in pairs and varied in shape, being round, slightly elongated, or irregular. Other strains had a similar form but the cocci were generally smaller, and a third group showed a greater proportion in short chains and the elements more elongated. The India ink preparations from the plain bouillon cultures also showed no definite capsules. In sugar bouillon Strain 156 gave a viscid growth, and when examined with India ink large distinct capsules were visible. None of the

other strains gave this type of growth in sugar bouillon nor did they show definite capsules. In regard to capsule formation, Schmitz (1912) states that the occurrence of capsules was not constant but sometimes they were present and under other conditions absent. Dible, and also Kendall and Haner mention that capsules were usually not demonstrable.

Various cultural reactions of these calf strains have been noted. In plain standard bouillon all the strains produced clouding and seven formed a viscid sediment rising in a spiral when the tube was shaken, and thus corresponding to Tricoire's bouillon growth. The other five strains formed a small amount of fine sediment, mixing easily when shaken. All strains coagulated milk. They all grew well in gelatin at room temperature and caused no liquefaction. The growth on potato was slight. On horse blood agar plates the strains produced deep colonies with small greenish zones and round smooth grayish surface colonies also with small zones of greenish discoloration. These differ from Dible's strains which he stated had no action on red blood corpuscles, but Dible's method for testing the action of the culture on blood was different, which may account for his result.

The fermentation capacity was tested on glucose, lactose, maltose, salicin, raffinose, sucrose, mannitol, and inulin. The result divided the strains into four groups. All strains fermented glucose, lactose, maltose, and salicin. Two cultures failed to ferment sucrose, and these same two and two others did not ferment raffinose but all four gave a positive reaction with mannitol. Seven other strains gave a negative result with mannitol and a positive reaction with raffinose. One strain fermented all the sugars including inulin. As already mentioned, the four groups agree with the type organism and variants I, IV, and V of Kendall and Haner. The majority of the calf strains correspond to their variant V. These groups with the pH values for the calf strains have been tabulated.

These results indicate that the calf enterococci correspond with certain types of the organism isolated from other sources. None of the calf strains in this collection agreed with variants II and III of Dible, or these same types plus variant VI of Kendall

and Haner. The pH values showed a fairly uniform amount of acid produced by the different strains in each sugar whenever fermentation occurred, except Strains 1075, 1082, and 1094, which formed less acid from raffinose than the other cultures which fermented this sugar. Strain 1106 agreed with the inulin-fermenting type of enterococci and differed from pneumococci

TABLE 1

Fermentation reactions of calf strains of enterococci grouped according to the types of Kendall and Haner

GROUPS		FERMENTATION REACTIONS WITH pH VALUES FOR CALF STRAINS							
Kendall and Haner	Calf strains	Glucose	Maltose	Lactose	Salicin	Sucrose	Mannitol	Raffinose	Inulin
Type culture				+	+	+	+	−	−
	1070c	4.8	5.0	5.0	5.0	4.9	5.3	7.6	7.6
	156	4.8	4.9	5.0	5.0	4.8	5.3	7.6	7.7
Var. I				+	+	−	+	−	−
	193	4.9	4.9	5.1	5.0	7.6	5.4	7.5	7.5
	945	4.8	5.0	5.0	5.0	7.6	5.4	7.6	7.6
Var. II				+	+	+	−	−	−
Var. III				+	+	−	−	−	−
Var. IV				+	+	+	+	+	+
	1106	4.8	4.8	5.0	5.0	4.8	5.2	5.0	4.8
Var. V				+	+	+	−	+	−
	1070 (2nd)	4.8	4.9	5.0	5.0	4.9	7.6	4.8	7.5
	1072	4.8	5.0	5.0	4.9	4.9	7.6	4.9	7.5
	1074	4.8	4.9	5.0	4.9	4.8	7.6	4.8	7.5
	1121	4.9	4.9	5.0	4.9	5.0	7.6	4.8	7.5
	1075	4.8	5.0	5.2	5.1	4.9	7.7	5.8	7.6
	1082	4.8	5.0	5.1	5.0	4.8	7.7	5.5	7.6
	1094	4.9	5.0	5.1	5.0	4.9	7.7	5.7	7.6
Var. VI				+	+	+	−	+	±

in being insoluble in bile. All the other strains of enterococci were also insoluble in bile.

In experiments on heat resistance 24-hour bouillon cultures in amounts of 2 cc. were heated in corked tubes in a water bath at 60°C. for 30, 15, and 5 minutes. One cc. of the heated culture

was plated and one cc. added to fresh bouillon. After the cultures had been heated for 30 minutes, six developed clouding in bouillon and showed plates filled with colonies. Another strain resisted heating at 60°C. for 15 minutes, and two others showed a few resistant organisms after 15 minutes exposure in one experiment and none in another; while three strains were consistently sensitive to heat at 60°C. for 15 minutes but withstood 60°C. for 5 minutes. It was noted that the seven strains forming a ropy sediment in bouillon were the most heat resistant. Dible considered heat resistance a character differentiating enterococci from streptococci. He stated that all enterococci in broth cultures survived heat at 60°C. for 15 minutes while streptococci were killed in 5 minutes and that he found only one exception to this rule. According to him, the property of heat resistance was due to the presence of some specially resistant cells since most of those present in the culture were killed.

The limiting and optimum H-ion concentrations were determined by the same method as that described in the paper on *B. acidophilus* except that ordinary standard agar was used for plating instead of agar adjusted to pH 6.8. Three strains were used, 1082, 1106, and 193. The growth after 24 hours changed the initial pH toward the acid side, probably because traces of muscle sugar were present. The organisms were able to grow in an alkaline medium of pH 9.0 and certain strains at a slightly higher pH. No growth occurred in bouillon with a pH value of 10.0. The optimum for all the strains tested was between pH 8.0 and pH 6.0. The acid limit varied with the individual strains. Two cultures multiplied at pH 5.0 but gave no growth at pH 4.8, and one strain failed to grow in bouillon of pH 5.5.

Three sets of mixed culture experiments were made in which the enterococci were grown with *B. coli*, with *B. acidophilus*, and finally with both of these organisms. Plain and glucose bouillon were inoculated with the mixtures and a pure culture of each reserved as a control. The strain of *B. coli* used was one isolated within the year from a case of scours. Colony counts were made to determine the number of organisms inoculated and the subsequent growth occurring in the tubes. In the first

experiment with a mixture of enterococci and *B. coli*, the results showed that in the glucose bouillon both organisms grew abundantly in the first 24 hours. The pH value reached 5.0 on the 2nd day. From the 3rd day on the enterococcus kept the ascendency over *B. coli*. In the plain bouillon tube the two strains multiplied together to about the same degree and at the end of a week both were living in large numbers. The pH at this time was 7.8. Altogether the results indicated that the enterococcus is more resistant to acid than *B. coli*.

Experiments in which enterococci were mixed with *B. acidophilus* were carried out in plain and glucose bouillon of three different pH values,—namely, pH 7.4, 6.8 and 5.5. The mixed growth was controlled by pure cultures of each organism. The development was determined by noting the clouding of the bouillon, examining stained preparations microscopically, and making colony counts. The results showed that in bouillon of pH 7.4 and 6.8 the enterococci always grew very abundantly in the beginning, clouding the tubes in 4 to 5 hours, while *B. acidophilus* showed no visible growth at this time. After 24 hours the acidophilus control tubes were well clouded, indicating that *B. acidophilus* grows well in this medium if it is given time to develop without interference. The mixed culture tubes at this time contained chiefly enterococci. The results were similar in both plain and glucose bouillon. These pH values of 7.4 and 6.8 were within the range of optimum growth for enterococci which gave them the advantage. On the other hand, *B. acidophilus* is generally more resistant to acid than enterococci. In the tubes containing bouillon of pH 5.5 the enterococcus growth was delayed. This H-ion concentration is beyond its range of optimum growth but within that for *B. acidophilus*. In this series of tubes no visible clouding occurred within 4 to 5 hours, but after 24 hours all the cultures were clouded. The mixed culture showed acidophilus bacilli present in numbers equal to or slightly above those of the enterococci. This is in contrast to the development in tubes were the enterococcus multiplication was not delayed. However, after 48 hours the plain bouillon tubes in the pH 5.5 series showed a continued increase of enterococci resulting

in their predominance in the mixed culture; while in the glucose bouillon mixed culture of this series according to stained preparations the enterococci did not predominate but were held in check, probably by the increased acidity. Altogether the results indicate that the enterococcus can prevent the growth of *B. acidophilus* by means of its more rapid multiplication, and that the acid reaction produced in the glucose tube has little effect on the development whenever the early rapid growth of the enterococcus can take place, for the multiplication of *B. acidophilus* was interfered with in both the glucose and plain bouillon tubes of an original pH of 7.4 or 6.8. However, the acid formed in these tubes tends in the end to favor *B. acidophilus* against enterococci, since acidophilus bacilli are slightly more resistant to acid, and in a few cases *B. acidophilus* has been found persisting in such cultures after 3 or 4 days. On the other hand, if the original acidity is beyond the range of optimum growth for the enterococci and within that for *B. acidophilus*, then the enterococcus growth is delayed and *B. acidophilus* has a chance to multiply. Thus two of the factors influencing the growth and final outcome of a mixture of enterococci and *B. acidophilus* are (1) that of the rapid growth of the enterococci, which is favored or interfered with by the original pH value of the medium, but which in itself is harmful to be development of *B. acidophilus*; and (2) the acidity produced in the glucose bouillon, which in the end tends to favor *B. acidophilus* against enterococci.

The third type of mixed culture experiment, in which enterococci were mixed with both *B. coli* and *B. acidophilus*, agreed in its results with the other two groups of experiments. In this case, in the glucose bouillon both enterococci and *B. coli* multiplied greatly in the beginning. The pH reached 5.2 in 24 hours and 5.0 on the 2nd day. *B. coli* was the first organism to disappear and it was survived by the enterococci and *B. acidophilus*. The growth of *B. acidophilus* was checked in the beginning, for it was not recognized in the plate cultures during the first 4 days, but on the 8th day it was growing in the glucose bouillon culture. In the plain bouillon tube *B. acidophilus* was not recognized in plate cultures after the first 24 hours, but *B. coli*

and the enterococci grew well together and at the end of a week both were living in large numbers. The pH of this culture ranged from 7.1 to 7.7. All the mixed culture experiments bring out the effect of the H-ion concentration in relation to the action of these strains against each other. They show that both enterococci and B. acidophilus are antagonistic to *B. coli* by their greater resistance to acid; but enterococci are antagonistic to *B. acidophilus* because of their multiplication in greater numbers, and the acid reaction produced by the two strains in mixed culture in glucose bouillon tends in the end to favor *B. acidophilus* against enterococci.

CONCLUSIONS

1. Enterococci were found to exist in the digestive tract of normal calves. They were also present in varying numbers in calves suffering from diarrhea, or scours. They sometimes developed in practically pure culture at certain levels.

2. The enterococcus organisms from calves do not represent a homogeneous group but are separated into four divisions according to fermentation reactions. These groups agree with the type form and certain variants described by Dible and by Kendall and Haner.

3. All the calf strains examined agreed in coagulating milk, leaving gelatin intact, producing green zones on blood agar plates, fermenting glucose, lactose, maltose, and salicin, and maintaining the same range of H-ion concentration for optimum growth. They differed in the fermentation of mannitol, sucrose, raffinose, and inulin; also in capsule formation, in the character of sediment in bouillon, in the degree of resistance to heat, and in the limiting H-ion concentration.

4. Enterococci growing in mixed cultures with *B. coli* are able to survive *B. coli* whenever acid conditions are produced. Enterococci are also harmful to *B. acidophilus* when they are grown together in mixed cultures, unless the early development of the enterococcus is delayed. In this case the injurious effect is not due to acid but rather to the rapid multiplication of the enterococci. Whenever acidophilus bacilli survive in such a mixed

culture they are found later in the tubes containing acid; thus the acidity tends in the end to favor *B. acidophilus* against enterococci.

5. The enterococcus differs from *B. acidophilus* in its ability to grow in a more alkaline medium, and also in being slightly less resistant to acid, although it is more resistant than *B. coli*.

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THE USE OF POTASSIUM TELLURITE IN DIFFERENTIAL MEDIA[1]

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The chemical and pharmacological properties of tellurium have long been a matter of interest to investigators. Early work groups tellurium and the closely related substance, selenium, with arsenic and antimony in their effect on the mammalian organism. These substances belong to the intermediate group of elements, which are capable of functioning as bases or as non-metals. Tellurium, like selenium, when it is a base, is usually bivalent (H_2Te), but in tellurious acid it is tetravalent (H_2TeO_3), and in telluric acid, hexavalent (H_2TeO_4). A resemblance can be seen to the similar compounds of sulphur, to which tellurium and selenium are closely related chemically and physically.

Gies, and Mead and Gies, in 1901, reviewed earlier work and discussed the physiological and toxicological effects of tellurium in detail. They found that tellurites were readily reduced to metallic tellurium by living protoplasm, and that granular deposits of the metal were widely found in the tissues of animals killed by the administration of toxic doses of tellurite.

Knop (1885) and Bokorny (1893) found that sprouting maize, algae and infusoria are uninjured by concentrations of potassium tellurite as low as 0.1 per cent. Jones (1909), in a study of selenite, found that heated tissue does not lose its ability to reduce the salt, and concluded that the active agent was a reducing sugar. He demonstrated *in vitro*, that glucose and related reducing substances readily caused the precipitation of metallic selenium at 35°C. and higher temperatures.

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Scheurlen (1900) and Klett (1900) extended the study to bacteria. Scheurlen found that *B. anthracis* and other bacteria were colored by reduced metal when cultivated in the presence of salts of tellurious and selenious acids. Klett studied the growth of numerous bacteria and of molds under the influence of compounds of selenium and tellurium. He found that the development of molds and of certain bacteria was not materially affected by the presence of small quantities of sodium tellurite, while that of certain other organisms was strongly inhibited. Some types seemed more susceptible to tellurites, some to selenites. This author also noted the reduction phenomenon, and stated that the formation of intracellular granules was proportional to growth. He found that selenate was relatively inactive and concluded by analogy that tellurates would be less active than tellurites.

Klett (1900) mentions *B. diphtheriae* as one of the organisms employed by him in his study. In the quantities that he used (not measured accurately), growth was slower than in controls, but after several days a reduction of the salt was observed. However, many other organisms were much more strongly inhibited.

Conradi and Troch, in 1912, attempted to use these salts for differential purposes. A modification of Loeffler's serum medium containing a small quantity of calcium malate and potassium tellurite (Te_4 concentration $\pm$ 1:10,000) was used. This medium is stated to be inhibitive for many saprophytes, and to hold overgrowth effectively in check. In addition, the colonies of *B. diphtheriae* and related organisms are easily selected, since they are strongly reductive. These authors found the blackening, although not specific, to be a characteristic feature at the close of the usual incubation period (eighteen to twenty-four hours), when colonies of other types usually failed to show reduction.

Smith, in 1914, compared the differential antiseptic action of potassium tellurate (Te_6 $\pm$ 1:18,800), and tellurite. He found the action of the latter to be less marked. This conclusion does not seem to have been confirmed by others, the weight of evidence being in favor of the greater activity of tellurites. The phenomenon of reduction was not emphasized by this medium, as

the author reported that the colonies of *B. diphtheriae* were white. The basic medium is an agar containing 5 per cent of serum. Smith states that this quantity is the optimum, since the use of a smaller amount gave less vigorous growth, while more decreased the differential antiseptic effect of the salt.

In 1921, Joachimoglu and Hirose determined the concentrations of the tellurates, tellurites, selenates and selenites of sodium tolerated by *B. diphtheriae*. An emulsion of organisms grown on Loeffler's medium was inoculated into tubes of glucose broth containing varying dilutions of these salts, and after twenty-four hours at 18 to 22°C., transfers were made to a suitable medium. The following concentrations were destructive under these conditions: Te_4 (as tellurite) 1:420; Te_6 (as tellurate) 1:125; Se_4 (as selenite) 1:1160; Se_6 (as selenate) 1:666. The comparatively high concentrations of these salts, tolerated by *B. diphtheriae* are in marked contrast to those effective for the destruction of many of the organisms tested, and this fact gives an explanation of the differential value of compounds of selenium and tellurium. An illustration of this wide difference is seen in the comparison of the effect of tellurite on *B. diptheriae* and *B. typhosus*. The former tolerates a concentration of tellurium about 4000 times greater than the latter. On examination of the figures given above, it will be noted that, contrary to the experience of these authors with most of the organisms tested by them, selenium is more toxic than tellurium for *B. diphtheriae*.

Douglas (1922) reports excellent results in the isolation of *B. diphtheriae* on a plating medium consisting of a trypsinized serum agar containing tellurite (Te_4 1:6000). He employs 10 to 15 per cent of a serum of which the antitryptic activity has been previously neutralized by the addition of trypsin. On plates examined at the end of eighteen to twenty hours, *B. diphtheriae* and related organisms show a very characteristic darkening and a granular appearance. Few other organisms found in throat cultures give a similar picture at this stage.

Megrail (1923) has found a serum agar containing 1:20,000 potassium tellurite very satisfactory for the isolation of diphtheria bacilli.

Pergola (1921; 1922; 1923) is reported as using several combinations of potassium tellurite with agar, gelatin, serum, etc. He also employs, as accessory to his plating media, an enrichment serum broth containing tellurite. Since the original articles are not available it is impossible to examine his methods in detail. However, the conclusions state that tellurite is effective as a selective, inhibitory agent, and that the use of tellurite media permits the isolation of *B. diphtheriae*, even when present in small numbers. The author states that selenium cannot be used as a substitute.

Greenspon (1923) and Hill (1922) have recently advocated the use of a moderately acid reaction (pH 6.6±) as favorable for *B. diphtheriae* and inhibitive for other organisms.

This review of the literature indicates the marked value of certain tellurium compounds in differential media, especially when used for the isolation of diphtheria bacilli. As media containing tellurites have not been extensively used in routine laboratory work in this country, it has been thought that a report of our experience with a medium similar to those recommended by Douglas (1922) and Smith (1914) might be of interest.

The routine procedure that had been employed in this laboratory to isolate *B. diphtheriae* for the purpose of determining virulence was as follows: A representative portion of the original growth on Loeffler's medium was emulsified in 20 per cent ascitic-fluid broth, and the emulsion plated in series on two plates of medium containing 2.5 per cent agar, 0.2 per cent glucose, and 5 per cent horse serum. The plates and the slanted tube were incubated. The tube of broth served as an enrichment medium, its value resting on the fact that if diphtheria bacilli are present a pellicle may be formed. After eighteen to twenty hours, another plate culture was made from this enrichment broth, a fragment of the pellicle being used, if present, or if not, a loopful of broth, taken from the surface region. This plate was fished on the succeeding day if the first plates had not permitted the isolation of *B. diphtheriae*. The colonies on this plating medium were small, but were much more readily fished than from a similar medium without serum.

Since the plating medium used in this laboratory approximates the basic media of Smith (1914) and Douglas (1922), it was used as the starting point for this study.

Tests were made to determine the optimum amount of tellurite—that which would permit the maximum inhibition of other organisms without decreasing the size of the *B. diphtheriae* colonies—and to determine whether or not tellurite, in the concentration selected, affects the virulence of the strains isolated.

A few tests were made to gain preliminary data, the results of which may be summarized as follows:

1. Tellurium in concentrations of 1:34,000 and 1:36,000 permits satisfactory growth of *B. diphtheriae* on an infusion glucose-serum agar base, while 1:20,000 and 1:22,000 concentrations are somewhat inhibitory.

2. Colonies of *B. diphtheriae* on media containing 1.5 per cent agar are more characteristic than those on the routine 2.5 per cent agar plating medium. The heavier agar had been used in the routine since it tended to inhibit the rapid spread of motile, contaminating organisms.

3. Colonies of *B. diphtheriae* are fairly distinctive on this medium, being small, granular and "smoky" after eighteen to twenty-four hours' incubation.

A supply of 1.5 per cent agar with 5 per cent horse serum and 0.2 per cent glucose was prepared with approximately 1:8000, 1:17,000, 1:34,000, 1:50,000, 1:66,000 and 1:100,000 parts of tellurium.

No growth of *B. pyocyaneus* or *B. subtilis* was obtained on any of these media after twenty-four hours' incubation. Three cultures of virulent diphtheria bacilli and a culture of *Staphylococcus aureus* grew well on all of the plates. The colonies of the diphtheria bacilli were smaller on the medium containing 1:8000 tellurium and in some cases on that containing 1:17,000. On all of the other plates the colonies were of approximately the same size. More blackening occurred, however, on the medium containing lower dilutions of tellurite.

It was found that the medium, when prepared as described, frequently became contaminated with molds. As previous work

had shown that a tellurite solution cannot be sterilized by heat, it was decided to sterilize the solution by filtration. A supply of potassium tellurite solution, 1 per cent by weight, approximately 0.58 per cent by actual test, was therefore prepared. This solution was passed through a Mandler filter. Chemical tests showed the solution tube very slightly concentrated after the filtration, in one determination it being 0.577 per cent before passing through the filter and 0.583 per cent after filtration. Media prepared with the filtered solution proved satisfactory.

After these preliminary tests it was thought advisable to compare the efficiency of the medium containing tellurite with that previously employed in the routine for the isolation of

TABLE 1

*Results obtained with both routine and tellurite media from March 18 to August 18**

	B. DIPHTHERIAE	B. DIPHTHERIAE LIKE	TOTAL
Isolated with tellurite plates only.....	22	6	28
Isolated with routine plates only.....	12	9	21
Isolated with both.....	43	16	59
Unsuccessful with both.....	0	0	25
Total.....	77	31	133

* In case atypical organisms are found in cultures submitted for routine examination, an attempt is made to identify the diphtheria-like bacilli. From many of such cultures nothing of significance is isolated.

diphtheria bacilli from throat cultures. The two media differed only in that the tellurite medium contained 1.5 per cent agar and approximately 1:17,000 parts of potassium tellurite, while the routine medium contained 2.5 per cent agar. The results of examinations during a period of five months are shown in table 1.

In 24 of the 43 cases in which diphtheria bacilli were isolated from both routine and experimental media, the percentage of successful fishings was higher with the experimental media; in 10 cases the percentages were equal.

So far as can be determined, the presence of potassium tellurite in the medium has no appreciable effect on the virulence of the diphtheria bacilli isolated. Of the 22 strains isolated on the tellurite medium 17 proved to be virulent and 5 proved to

be non-virulent. Thirty-four cultures that had been isolated on the routine medium and found to be virulent were replated on a medium containing approximately 1:34,000 parts of tellurium. Colonies were fished after twenty-four hours and the virulence test was repeated. All of these cultures were still virulent.

Colonies of *B. diphtheriae* on the tellurite medium are not difficult to distinguish and can be fished microscopically with success when they are present in moderate numbers. They are white, or white about the periphery with grey centers, the depth of color increasing with the period of incubation up to forty to forty-eight hours. Staphylococci are entirely black, or black in the center with a narrower light periphery. Microscopically, the diphtheria colonies are round, coarsely granular, with dull, dark or "smoky" raised centers. The periphery is flat and lighter with unbroken or very slightly irregular edges. Microscopically, colonies of staphylococci are of about the same size or somewhat larger than colonies of *B. diphtheriae*, but are black, highly refractive, round, smooth and raised, with edges entire. Colonies of *B. hoffmanni* resemble those of *B. diphtheriae* but have a heavier periphery. Streptococcus colonies are not always characteristic. Microscopically, they may be confused with those of *B. diphtheriae* as they are white, or white with greyish centers. They are smaller, however, and microscopically are flat with a slight brownish coloration of the center.

There are some other factors which should be considered in a study of this sort, such as the keeping quality of the medium and of the stock tellurite solution. Smith (1914) states that tellurate gradually loses its antiseptic action. The ease with which reduction occurs suggests that the tellurite solution is relatively unstable. We have found, however, that a medium containing tellurite solution that had been filtered through a Mandler filter twenty days before use proved entirely satisfactory. Medium in plates that has been left eight days at a temperature of 3° to 6°C. has proved somewhat less satisfactory than that freshly prepared, the colonies of diphtheria bacilli being somewhat smaller on the former medium. Growth of *B. pyocyaneus* and *B. subtilis* was completely inhibited on it however. The smaller

size of the colonies of diphtheria bacilli may have been due to the fact that the surface of the medium that had been stored was dryer than in the case of that which was freshly prepared.

TABLE 2

Growth of different organisms on medium containing tellurite 1:34,000

LITTLE OR NO INHIBITION	SLIGHT INHIBITION	MARKED INHIBITION	NO GROWTH
Streptococcus hemolyticus	Pneumococcus Type I	*Micrococcus meningitidis*	*Micrococcus catarrhalis*
Staphylococcus aureus	Pneumococcus Type II	*Sarcina lutea*	*Micrococcus gonorrheae*
Micrococcus tetragenus	Pneumococcus Type III	*Bacillus dysenteriae*, Shiga	*Bacillus abortus*
Bacillus acidophilus	*Bacillus cholera suis*	*Bacillus dysenteriae*, Flexner	*Bacillus anthracis*
Bacillus coli	*Bacillus morgani*	*Bacillus dysenteriae*, Mt. Desert	*Bacillus anthracis symptomaticus*
Bacillus diphtheriae	*Bacillus mucosus capsulatus*	*Bacillus enteriditis*	*Bacillus botulinus* A
Bacillus hoagii	*Bacillus pertussis*	*Bacillus lactis aerogenes*	*Bacillus botulinus* B
Bacillus hoffmanni	*Bacillus rhinoscleroma*	*Bacillus paratyphosus* A	*Bacillus fecalis alkaligenes*
Bacillus influenzae		*Bacillus paratyphosus* B	*Bacillus fluorescens*
Bacillus proteus vulgaris		*Bacillus pestis caviae*	*Bacillus histolyticus*
Bacillus proteus X19		*Bacillus suipestifer*	*Bacillus mallei*
Bacillus welchii		*Bacillus typhosus*	*Bacillus melitensis*
Bacillus xerosis			*Bacillus oedematis maligni*
Spirillum cholera			*Bacillus prodigiosus*
Actinomyces bovis			*Bacillus pullorum*
Aspergillus niger			*Bacillus putrificus*
Saccharomyces cerevisiae			*Bacillus pyocyaneus*
			Bacillus sporogenes
			Bacillus subtilis
			Bacillus tetani
			Bacillus typhii murium
			Bacillus violaceus

After making the tests with diphtheria bacilli, it was thought well to learn with what other types of organisms the medium

would be useful. A number of cultures from the stock collection were tested on a medium containing approximately 1:34,000 tellurium. Table 2 shows the character of the growth obtained (for *B. influenzae* and *B. pertussis* blood extract agar was employed). These results indicate that media containing potassium tellurite may be valuable in the isolation of Gram-positive cocci, certain of the higher bacteria, molds and yeasts, and possibly in the isolation of *B. welchii* from mixtures with some of the other anaerobes.

The method of preparing the medium is as follows:

Beef infusion agar 1.5 per cent	1 liter
Horse serum (sterile)	5 per cent or 50 cc. per liter
Glucose C. P., 20 per cent solution (sterile)	1 per cent or 10 cc. per liter
Potassium tellurite C. P., 1 per cent solution (0.58 per cent on test)	1 per cent or 10 cc. per liter 1:34,000 Te_4 (1-17,000 K_2TeO_3)

Procedure. Melt the agar and cool to 50°C. Add the serum glucose and tellurite solutions, all previously warmed to 50°C. Mix well and insert a sterile siphon. Dispense in Petri dishes. Incubate the plates and examine for contaminations before use.

The solution of potassium tellurite is prepared as follows:

Potassium tellurite C. P.	0.25 gram
Distilled water	25.0 cc.

Procedure. Weight 250 mgm. K_2TeO_3 on a chemical balance. Grind to a very fine powder in a small mortar and add about 10 cc. of the water. Mix well, allow the undissolved portion to settle and pour off the supernatant fluid. Add more of the water to the residue, grind again and combine the two portions. Rinse the mortar with the remainder of the water.

The solution is then passed through a paper filter and then through the Mandler to sterilize it.

Note. It is difficult to get this compound into solution and even after thorough grinding in the mortar, a small amount of fine suspension is visible. Heating gently to about 55°C., aids the action somewhat.

As was indicated in the preliminary tests the range of the tellurium content can be wide. It has been thought advisable,

however, to determine the approximate amount of the salt that will dissolve, especially when a new supply is purchased. The method described by Gooch (1912) and Gooch and Donner (1892) has proved satisfactory.

SUMMARY AND CONCLUSIONS

A concentration of potassium tellurite approximating 1:17,000 gave excellent results in the isolation of diphtheria bacilli from throat cultures, and seemed to have no effect upon the virulence of the organisms. From the observation of the growth of a number of cultures in the stock collection, it was found that certain Gram-positive cocci, some of the higher bacteria, yeasts, and molds seemed to be inhibited only slightly if at all, while many of the motile bacilli, including a number of anaerobic organisms, were markedly inhibited by potassium tellurite in the concentration mentioned.

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